EXAMINING GLUCOSE CONTROL AND WORKING MEMORY AS A FUNCTION OF AGE IN OLDER TYPE 2 DIABETICS: RESULTS FROM THE LOOK AHEAD BRAIN MRI ANCILLARY STUDY

by

Regina L. Leckie

B.S., Denison University, 2008

Submitted to the Graduate Faculty of

The Dietrich School of Arts and Sciences in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2016

UNIVERSITY OF PITTSBURGH

THE DIETRICH SCHOOL OF ARTS AND SCIENCES

This dissertation was presented

by

Regina L. Leckie

It was defended on

April 11th 2016

and approved by

Kirk Erickson, Department of Psychology

John Jakicic, Department of Health and Physical Activity

Peter Gianaros, Departments of Psychology and Psychiatry

Michele Levine, Departments of Psychology and Psychiatry

Natasha Tokowicz, Department of Psychology

Dissertation Director: Kirk Erickson, Department of Psychology

EXAMINING GLUCOSE CONTROL AND WORKING MEMORY AS A FUNCTION OF AGE IN OLDER TYPE 2 DIABETICS: RESULTS FROM THE LOOK AHEAD BRAIN MRI ANCILLARY STUDY

Regina L. Leckie, PhD

University of Pittsburgh, 2016

Type 2 diabetes mellitus (T2DM) is becoming increasingly prevalent in the US, especially in older adults. In fact, 25.9% of adults over the age of 65 have been diagnosed with T2DM. In addition to the increased cardiovascular disease risks, cognitive and brain health impairments are also associated with T2DM. Overall lower brain volumes and poorer memory function is observed in T2DM, indicating poorer brain health than non-diabetics. While the mechanisms behind poorer cognitive function and brain health are unknown, impaired glucose control, as measured by glycated hemoglobin (HbA1c), is one aspect of T2DM that may provide insight into this ongoing inquiry. The Action for Health in Diabetes (Look AHEAD) trial was designed to longitudinally examine the cardiovascular health effects of weight-loss in older adults diagnosed with T2DM. The trial randomized participants into two conditions: Intensive Lifestyle Intervention (ILI) consisting of a physical activity regimen and caloric-restriction diet, and Diabetes Support and Education (DSE) control group offering seminars on diet, exercise, and diabetes symptoms management. At year 10 of the trial, participants ($N = 237$, mean age = 67 years) were recruited for a magnetic resonance imaging (MRI) ancillary study, in which participants completed a working memory task during a functional MRI (fMRI) scan and blood draw to measure HbA1c levels. Results indicate that age moderates the relationship between intervention group and cognitive function and the relationship between HbA1c and cognitive function, such that older adults in the ILI group, but not control group have the best glucose control yet worst performance. Further analyses

reveal that the older adults in the ILI group with high HBA1c levels performed significantly better than those with lower HbA1c levels. Together, these results suggest that HbA1c is protective to working memory function in old age within T2DM. fMRI identifies regions in the prefrontal cortex, cingulate, insula, and hippcampus where HbA1c is associated with brain activity during the working memory task. However, no group differences in brain activity were identified. As T2DM becomes more prevalent in the population, it is critical to understand the cognitive impact this disease may have on patients, especially as they age.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1.0 INTRODUCTION

Every year, the number of individuals diagnosed with Type 2 diabetes (T2DM) in the United States rises, with over 9% of the population currently diagnosed [3]. This trend follows the rise in obesity in the United States as its symptoms, such as poor glucose control, are diagnostic criteria in T2DM. In fact, an obese individual is at a 43% increased risk of developing T2DM than a non-obese individual [3]. Older adults are also the highest percentage of individuals diagnosed with T2DM and as the baby boomers continue to age, the older adult T2DM population increases. Aside from the many cardiovascular health risks that are associated with T2DM, type 2 diabetics are also at a greater risk for the development of dementia [48], such as Alzheimer's disease [31, 38], and cognitive impairment [65, 110]. Even more disturbing, is that throughout the lifespan, individuals with T2DM display poorer overall cognition than their age matched peers [11], and at rates that surpass obesity related effects [111]. The question is then raised, what is different about T2DM that could cause the observed cognitive impairment and poorer brain health in older adults? One potential answer is the unique lack of glucose control in T2DM; without proper glucose control and control through insulin signaling pathways, neurons in the brain are starved of glucose, causing impaired function and long-term cell death [5]. However, the brain is a dynamic organ and can adapt to function in adverse situations, as seen in recovery of function after stroke or traumatic brain injury [98]. Yet, the impact of glucose control on brain function remains to be explored in this population.

While cardiovascular effects, such as hypertension, are traditionally credited as mediating obesity-related cognitive deficits [2, 13, 40, 111], several empirical studies [23, 30, 102, 103, 105] reveal a compelling hypothesis that insulin resistance and poor glucose control may be the cause for the marked cognitive impairment seen in T2DM. Insulin resistance is the biolog-

ical insensitivity to insulin, thought to be caused by chronic high levels of circulating glucose [52]. Glucose control is the ability to metabolize glucose, removing it from the bloodstream, where poor glucose control results in hyperglycemia (too much glucose) or hypoglycemia (too little glucose). Therefore, better glucose control is associated with less insulin resistance. Despite the fact that the mechanisms of insulin resistance are well established at the cellular level in relation to cardiovascular disease and T2DM in the periphery [29, 64, 89, 97], its implications in the brain in relation to cognition are still under investigation [10, 25, 44]. Additionally, the role of hyperglycemia and poor glycemic control on brain function remains unclear. Higher blood glucose levels, or glycated haemoglobin (HbA1c) levels, are associated with poorer cognition across multiple domains, yet the underlying mechanisms are unknown [11]. In fact, a recent meta-analysis found HbA1c levels to be associated with cognitive function, accounting for 10% of the variance in cognitive performance among T2DM patients [41]. Yet, large clinical trials have failed to find any cognitive benefits of lowering HbA1c on cognitive performance in older T2DM patients [60]. This suggests that the role of HbA1c in cognitive function may be different in older adults and that age may also play a part in the relationship between T2DM and cognition. Additionally, this might also suggest that HbA1c is not the sole mechanism behind diabetes-related cognitive impairment. However, there is currently not enough information or understanding of the mechanisms underlying the relationship between T2DM and cognition to confirm or deny any such claim. This highlights the importance of continued work to examine the relationship between T2DM and cognition.

1.1 GLUCOSE CONTROL

Glucose control is determined by the efficiency in which the body removes glucose from the bloodstream, transporting it into cells for use and storing the remainder. Insulin is a critical component of the uptake of glucose from the bloodstream into the cell, and is insufficient in T2DM. The biology of insulin's role in intercellular glucose transport in the body has been well established for decades [71]. In response to elevated blood glucose levels,

beta cells in the pancreas secrete the hormone, insulin, into the bloodstream. Insulin then binds to insulin receptors (INSR) on the cellular membrane, creating a signaling cascade that instigates glucose transporters on the outside of the cell to remove glucose from the bloodstream and transport it into the cell for use. Therefore, insulin is effective via binding to insulin receptors on the cellular membrane, creating a signaling cascade within the cell that facilitates glucose uptake via insulin-sensitive glucose transporters.

As with many aspects of biology, there are large individual differences in glucose control. For example, race plays a role in glucose control in non-diabetics, as non-hispanic African-Americans tend to have higher circulating glucose levels than non-hispanic Caucasians [46]. Because of these individual differences, there is a range of glucose control within the population. Several tests exist to determine an individual's level of glucose control. The most common measure is that of glycated haemoglobin, or HbA1c. HbA1c is measured based on the knowledge that when glucose is removed from the blood stream, it is no longer bound to red blood cells. Red blood cells have a lifespan of several months and can maintain a bond with glucose for that duration until it is removed via insulin signaling. When the hemoglobin in the red blood cell binds with glucose, it is glycated. Through a blood draw, the percentage of glycated red blood cells (those with glucose binding present) can be calculated. This percentage provides a snapshot into the last two to three months of a person's glucose control. The World Health Organization (WHO) has determined that individuals with an HbA1c of 7.0% or higher are considered to be diabetic [119]. This standard is global and does not vary across countries. Importantly, WHO states that levels less than 7.0% cannot rule out diabetes, and that other tests such as a fasting glucose tolerance test and an assessment of cardiovascular disease risk be administered to best diagnose T2DM. Therefore, it is possible to have an individual who is T2DM with both healthy ($< 7.0\%$) or unhealthy ($> 7.0\%$) HbA1c levels.

1.1.1 Glucose control and age

Glucose control also varies throughout the lifespan, resulting in more middle-aged and older adults being diagnosed with T2DM than adolescents and young adults. The Center for

Disease Control reported that adults between the ages of 45-64 had the highest diagnosis rate of T2DM in the USA, followed by adults 65 and older [18]. In fact, 25.9% of adults over the age of 65 have been diagnosed with T2DM. While many factors that contribute to a diabetes diagnosis, such as obesity, high cholesterol, and a sedentary lifestyle, increase with age, HbA1c also increases with age. Even in non-diabetics, HbA1c rises by 0.10% every decade [27]. This suggests that there is a possible relationship between glucose control and age. However, there is very little information on how advancing age affects HbA1c levels and what that means for T2DM patient's health. Addressing this gap in the literature, a study in the UK followed recently diagnosed T2DM adults over the age of 30 for 10 years [62]. The authors collected data from over 4,000 T2DM adults, mostly in their 60s at the start of the study, and tracked their HbA1c levels, as well as any changes in medication to better regulate glucose control. The authors report that HbA1c increased by an average of 0.047% annually in their participants and HbA1c average rose from 7.04% at 6 months after diagnosis to 7.49% at 10 years after diagnosis. While this is the only known study to examine long-term HbA1c changes in T2DM, it is important to note that HBA1c levels increased with age despite medication taken to control HbA1c levels.

1.2 GLUCOSE CONTROL IN THE BRAIN

Initially, the brain was hypothesized to be independent of the effects of glucose control and that it maintained its own stores and release of glucose in order to function. This theory was based on the thought that insulin could not cross the blood-brain barrier (BBB) [45]; however, many have refuted this; first, through demonstrating insulin transport through specific binding sites in the BBB in humans [80] and animals [32], and then by showing that peripheral infusions of insulin increase insulin levels in the cerebrospinal fluid [9, 101, 115] and brain tissue [8]. In fact, insulin transporters in the BBB are currently being used in experimental designs in primates to transport non-permeable enzymes across the BBB, allowing therapeutic pharmaceutical treatments to gain access to the brain [12]. Additionally, it was discovered that while insulin-insensitive glucose transporters, such as GLUT 1 and

GLUT3, exist in the brain, they are largely found on glial cells, leaving neurons to be largely insulin-dependent [88, 114]. The reliance on insulin for the uptake of glucose into neurons has led to the hypothesis that insulin resistance and poor glucose control would decrease neuronal glucose uptake, decreasing the neurons's ability to function properly and result in poorer cognitive function.

1.2.1 Possible mechanisms

In an effort to identify how glucose control would differentially affect brain function and resulting cognition, it is important to first take stock of the insulin receptors and insulin-dependent glucose transporters in the brain along with their functions. The main insulin-sensitive glucose transporters for neurons are GLUT4 and GLUT8 [14, 66, 88]. Insulin binding to INSRs and the resulting uptake of plasma glucose from the bloodstream by these transporters is critical for neuronal functioning [76], as it has been previously demonstrated that neurons do not maintain any internal glucose stores or reserves. In fact, glucose deprivation results in an increase in oxidative stress and neuronal death in the hippocampus [79]. Further, the cellular events associated with glucose deprivation closely resemble those of ischemic events, where ion flux cannot be regulated, resulting in increased intracellular calcium ion levels, neurotransmitter release, excitotoxicity and cell death [1]. Finally, insulin resistance and impaired glucose transport seen in T2DM have been associated with increased amyloid-beta plaques [28], a diagnostic marker of Alzheimer's Disease.

While the need for neurons to take in glucose is universal throughout the brain, demand and density of INSRs and insulin-sensitive glucose transporters varies. For example, some neurons rely on lactose stores in glial cells [85], such as orexin neurons in the hypothalamus, anterior cingulate cortex, and locus ceruleus [82], while others rely on insulin binding, such as those found in the dorsal root ganglion [35, 56]. Therefore, different brain areas may be more or less affected by glucose control, where some may function normally under low-glucose environments and others may function poorly in insulin resistant environments. This is an important distinction to make, as some cognitive domains are more or less affected in T2DM. Therefore, it is possible that the sensitivity to T2DM in working memory, for example, may

be a result of higher reliance on insulin in the regions of the brain associated with working memory function. For example, regionally specific brain activity during a working memory task that was correlated with glucose control would support this hypothesis.

1.2.2 Regional specificity: Impact of glucose control is not universal

Structurally, better glucose control is reliably associated with greater [51, 55] or improved [60] global brain volume. While the mechanism underlying this relationship remains under investigation, there is evidence in the T2DM literature that regional atrophy is associated with observed cognitive deficits [72]. Some regions associated with cognitive function and performance in T2DM are the hippocampus and fronto-parietal regions.

The hippocampus is a brain region located in the medial temporal lobe, known for its role in memory and learning, as evidenced by case studies of injury in humans [39] and rodents as well as functional imaging data [28, 58]. Due to its dynamic properties, the hippocampus is regarded as a plastic region of the brain, modifiable by several environmental variables such as physical activity [35], environmental enrichment [39], stress [67], and aging [118, 121]. In relation to T2DM, the hippocampus is an insulin-sensitive brain region, demonstrating increases in learning and neuronal growth in association with increased insulin sensitivity [103]. The hippocampus also expresses large quantities of insulin receptors and insulin-sensitive glucose transporters [66, 109], resulting in speculation that hippocampal-dependent cognitive function would show deficits in the face of T2DM. Supporting this argument, relational and working memory function has been suggested to be partially mediated by the hippocampus [33], but is also reliant on a network of brain regions in the frontal and parietal lobes.

Fronto-parietal regions that support cognitive function are also likely associated with T2DM and/or glucose control, but there is little literature explicitly testing this hypothesis. In rodent models, insulin binding in the cerebral cortex and hypothalamus has been shown to be higher than other midbrain structures, suggesting that there are increased insulin receptors in these regions [47]. Expression of insulin-sensitive glucose transporters, like GLUT4, have not been examined in the prefrontal cortex of rodents, however, neuroimag-

ing techniques, such as the positron emission tomography (PET) can be used to measure glucose uptake in the human brain, and magnetic resonance imaging (MRI) can be used to measure the amount of brain tissue in a given region [42]. It has been suggested that T2DM is associated with decreased grey matter volume in these regions [16] and that intranasal administration of insulin improves cognitive processes associated with these regions [59] in humans.

Another example using neuroimaging is Gonzales and colleagues [43], who conducted an fMRI study to determine if insulin resistance mediates variability in working memory brain activity in a non-diabetic population. The authors examined regional brain activation during a working memory task that correlated with body mass index (BMI). The authors found a region in the right parietal lobe as significantly associated with BMI such that as activation in this region increased, BMI decreased. To isolate the possible effect of insulin resistance, the authors then added insulin resistance to the regression model. Interestingly, all variance previously explained by BMI was accounted for by insulin resistance, so they concluded that insulin resistance is a possible mediator for BMI related effects on working memory function. It is important to note that the described studies are the few that exist on the relationship between glucose control, brain function, and cognition. This leaves a large gap in the literature and many unknowns.

A few of the unknowns of particular interest are exactly how glucose control may change the architecture of the brain, what makes one brain region more at risk of glucose-induced changes, and what mechanism underlies any changes made as a result of glucose and/or insulin levels.

1.2.3 Plasticity: Changing the brain with lifestyle

In addition to a lack of definitive evidence on the relationship between glucose control in T2DM and brain function, there are many inconsistencies in the literature. One potential cause for such heterogeneity is that research on T2DM infrequently accounts for comorbidities, such as obesity-related health issues and a sedentary lifestyle [70]. Lifestyle factors, specifically physical activity, can have a large impact on the brain, affecting brain volume,

perfusion, and cognitive function.

Physical activity is repeatedly associated with cognitive function and has been to topic of numerous reviews and of consistent positive effects, especially in older adults [21, 57, 61, 87]. For example, Erickson and colleagues [35] found that greater amounts of physical activity were associated with better spatial memory performance and increased hippocampal volume. Additionally, hippocampal volume partially mediated the relationship between aerobic fitness and memory performance, suggesting that the effects of fitness on hippocampal volume results in better cognition. In addition, several studies have supported the hypothesis that engaging in physical activity influences the function and integrity of the prefrontal and parietal regions regions that support working memory function. For example, Colcombe and colleagues [21] conducted a randomized clinical trial of physical activity and found that 6-months of walking exercise increased prefrontal cortex activity during an attentionally demanding task relative to their non-exercising peers. While the majority of research on the effects of physical activity and fitness on brain function and cognition focuses on non-diabetic older adults, there is some evidence that physical activity is effective in improving cognition in T2DM.

Interestingly, physical activity improves glucose control in T2DM. Toledo and colleagues [107] determined that after a 4-month physical activity intervention, participants had significantly lower blood sugar levels and better glucose control measures than the control group. This suggests that changes in glucose control may be partially mediating the benefits of physical activity on cognitive and brain outcomes. Yet, no empirical evidence currently exists to examine these relationships in a single sample. Additionally, light to moderate physical activity is associated with better overall cognition in T2DM and is suggested as a preventative therapy for cognitive decline associated with T2DM [20].

Caloric restriction is another lifestyle factor that can influence the brain, although the majority of available data derives from animal literature and is very difficult to generalize to human populations. In rodents, caloric restriction is associated with less plaque build-up in Alzheimer's models [84] and less brain atrophy [22] than free-feeding in animals. In fact, it is well known that caloric restriction enhances hippocampal function and is associated with longevity in adult rodents [83]. However, the impact of caloric restriction on human

brain function, particularly those with T2DM, remains unknown and unexplored. One major concern in generalizing animal data to humans is that caloric restriction in animals reduces intake further than that of typical human dieting. Even in primate models, it is difficult to generalize effects of a 30% caloric reduction in primates to that of humans, as it is highly unlikely that humans would faithfully reduce their intake to this degree [92]. Moving forward, it is important to fill these gaps to determine 1) how glucose control acts in the T2DM brain and 2) how lifestyle changes in T2DM may alter the effect glucose control may have on brain function in this at risk population.

1.3 TYPE 2 DIABETES AND COGNITION

Across the lifespan, individuals with T2DM perform worse on many cognitive tasks than non-diabetics. However, the types of cognitive tasks vary, and the exact scope of which cognitive domains affected is unclear. In an attempt to identify domain-specific effects of glucose control on cognition, Ryan and colleagues [94] conducted a study using the drug rosiglitazone, a pharmaceutical that improves glucose control via insulin binding. They conducted a 24-week rosiglitazone intervention with 145 individuals with T2DM. They administered multiple cognitive tasks measuring declarative memory, reaction time, verbal learning, attention, and psychomotor speed both before and after the 24-week period. Interestingly, they found that after 24 weeks, individuals with T2DM who were placed on the medication increased their performance on a paired associates learning task by 30 percent. No change was seen in the control group or for any other cognitive task. This result provides evidence that memory may be sensitive to the effects of glucose control.

One specific type of memory that is often linked with poorer performance in T2DM is working memory [69]. Working memory is a cognitive domain that is typically grouped under executive function, defined as active updating and maintenance of information with a limited capacity and requiring high attentional demands [6]. In addition, working memory has been credited in the consolidation of information from short-term to long-term memory [7].

There are a plethora of tasks and tests that have been reliably used to measure working memory, such as the Backwards Digit-Span Task, the N-back, and Sternberg task. The Digit-Span Task is part of the Weschler Memory Scale test (WMS), a common neuropsychological battery of memory tasks [116]. In the Digit-Span task, participants are read a string of numbers and asked to repeat the numbers in order. Participants are also asked to repeat the numbers backwards (i.e. 1-2-6-4 repeated as 4-6-2-1), increasing the difficulty of this task. After two successful completions, a number is added to the string of numbers until the participant can no longer repeat the string. Working memory is measured as the number of numbers successfully repeated during the backwards portion of this task.

The N-back task is a computer-based task where participants view a stimulus one at a time on a computer screen [53]. The Letter N-Back is a common N-back task, where the stimuli are lowercase letters. In this task, participants are instructed to respond whether or not the letter currently on the screen matches a previously displayed letter. This task is broken into two parts. In the 1-Back participants are asked if the letter currently on the screen matches the letter immediately before it (i.e. a a is a match, ab is not a match). In the 2-back, participants are instructed to match the current letter with the letter two previous (i.e. aba is a match, aab is not a match). This task requires the participant to constantly update the memory of which letter or letters were previously displayed, and therefore accuracy and reaction time during the 2-back task is considered an accurate and sensitive measure of working memory.

Neuroimaging studies have examined the neural correlates of the N-back task for about 20 years and have consistently associated brain regions where activity during the task is greater than at rest. To summarize this body of work, Owen and colleagues [77] generated a meta-analysis of all regions associated with the Letter N-back task, identifying lateral premotor, dorsal cingulate, dorsolateral prefrontal, ventrolateral prefrontal, frontal pole, medial posterior parietal, inferior parietal, and thalamus regions as common among the literature. However, in T2DM activity in these regions is less than in healthy controls [19]. In addition, activity is also correlated with performance, such that greater activity was associated with higher accuracy. Therefore, in T2DM it is possible that lower brain activity is resulting in poorer cognitive function.

In relation to glucose control, working memory performance has been associated with circulating glucose levels. Sommerfield and colleagues [99] examined the effects of hyperglycemia on several memory systems including working memory, using the Digit Span task. Utilizing a glucose clamp, the authors were able to manipulate glucose levels in the bloodstream of older adults with T2DM. The manipulation included both increasing glucose to a hyperglycemic level and maintaining glucose at a normal, euglycemic, level. The authors reported no significant effects of hyperglycemia on the Forward Digit-Span task, however a significant negative relationship on the Backwards Digit-Span, such that elevated glucose levels in individuals with T2DM performed significantly worse than those who had glucose levels within a normal range. These results suggest that the more cognitively demanding working memory task of repeating the numbers backwards is affected by available glucose.

The Backward Digit-Span task has also been associated with obesity in older men but not women [34], such that obese men perform worse on the task than non-obese men. Unfortunately, it is difficult to separate vascular comorbities in obesity from glucose control, as these individuals did not meet the diagnostic criteria of maintaining an HbA1c ¿ 6.5%. Additionally, this study examined older adults who may also show age related effects of cognitive function.

The largest effects of T2DM on cognitive function are seen in older adults. When reviewing the literature, Ryan & Geckle [93] argue that T2DM adults between the ages of 55-75 not only perform worse on cognitive tasks than non-diabetic or younger diabetic peers, but they have higher incidence rates of comorbities of diabetes that also affect cognition. Upon close investigation, hypertension, diabetes duration, and hyperinsulimia are all age-related and associated with poorer performance on memory tasks. However, the disentanglement of each comorbidity from effects of age and relative effect on memory has yet to be done. Interestingly, in their review almost two decades ago, Ryan & Geckle speculate that brain morphology could underlie the cause of cognitive impairment in older T2DM adults; however, this relationship remains largely unknown.

1.4 CURRENT STUDY

The Action for Health in Diabetes (Look AHEAD) study offers a unique way to examine the complex relationships between T2DM, working memory, and brain function. The Look AHEAD Study was an intensive lifestyle intervention with older (>55 years) T2DM patients, where a diet of reducing caloric intake and physical activity were highly encouraged, compared to a control group of diabetes support and education. In this sample, a significant relationship between intervention groups and cognitive performance was reported [36], such that individuals in the treatment group who were overweight, but not obese, performed better on overall cognitive tasks than overweight and obese individuals in the control group. This relationship can be further explored in the Action for Health in Diabetes Magnetic Resonance Imaging Ancillary Study, which included functional magnetic resonance imaging (fMRI) and working memory, via the N-Back task, testing in a subsample of the LookAHEAD sample. Using fMRI, working memory performance, and physical activity data, brain regions that may be most affected by the intensive lifestyle intervention can be identified.

The current literature leaves several unknowns that may be addressed with the LookAHEAD Brain MRI study sample. For example, the relationship between glucose control and cognition is tested in two ways; 1) does HbA1c predict working memory performance (N-Back accuracy and response time) and 2) are there regional differences in brain activation during working memory associated with HbA1c. These questions have been broken into four main aims, outlined below and visualized in Figure 1.

It is hypothesized that the intervention group would have significantly better glucose control (lower HbA1c), better working memory performance, and greater brain activity than the control group. The current study will examine the effects of the intervention on working memory performance in the MRI sample and the fMRI BOLD signal during the working memory task, determine if brain activity correlates with working memory performance, in order to determine the relationship between brain activity and behavior, determine which regions of the brain are associated with glucose control (HbA1c levels), and examine any relationship age may play as a moderator of any direct or indirect observed effect.

Aim 1: Examine the group effects of working memory performance. It is hypothesized

that participants in the ILI condition will display better performance on the N-back working memory task, as displayed by higher accuracy and lower response times than DSE participants. Age will also be modeled as a moderator to determine if the oldest adults in the ILI group show perform significantly better than the oldest participants in the DSE group.

The next step of this aim is to identify any group effects of brain function during the working memory task. It is hypothesized that participants in the ILI condition will show higher percent signal change in regions of the brain associated with working memory (anterior cingulate cortex, fronto-partietal regions, hippocampus) than DSE participants during the higher cognitively demanding 2-back task, as seen in the 2-back > 1-back contrast.

Aim 2: Determine the relationship between working memory brain function and performance. I hypothesize that there is a significant relationship between brain function and cognitive performance, such that N-back accuracy will significantly correlate with BOLD activation in regions previously associated with working memory. Specifically, higher activation is anticipated in the dorsolateral perfrontal, posterior partietal, lateral premotor, and frontal pole regions. These regions are identified by Owen and colleagues [77] who performed a meta-analysis on common brain regions associated with the N-back task Figure 2.

Aim 3: Examine group effects of glucose metabolism. It is hypothesized that, as a result of increased physical activity and lower body weight, participants in the ILI condition will display significantly lower HbA1c levels than DSE participants, demonstrating better glucose metabolism. Age will be modeled as a moderator to determine if typical increases in HbA1c, as seen in age, are present in the DSE group, but absent in the ILI group.

The second part of this aim is to identify any group effects of working memory brain function associated with glucose metabolism. It is hypothesized that HbA1c levels will also significantly correlate with higher BOLD activation in previously described regions of the brain that are associated with working memory performance.

Aim 4: Determine if glucose metabolism mediates working memory performance. It is hypothesized that HbA1c levels mediate the group effects on N-back performance, suggesting that the ILI group performed significantly better on the working memory task than DSE participants because of their improved glucose metabolism. Age will be included as a moderator, as it is hypothesized to moderate the direct effect of group on performance and

group on HbA1c levels, such that this relationship is strongest in older adults.

Secondly, I will determine if glucose metabolism mediates working memory brain function. It is hypothesized that HbA1c levels mediate the group effects on BOLD activation during the N-back task, suggesting that glucose metabolism affects cognitive performance by enhancing activation in related brain regions.

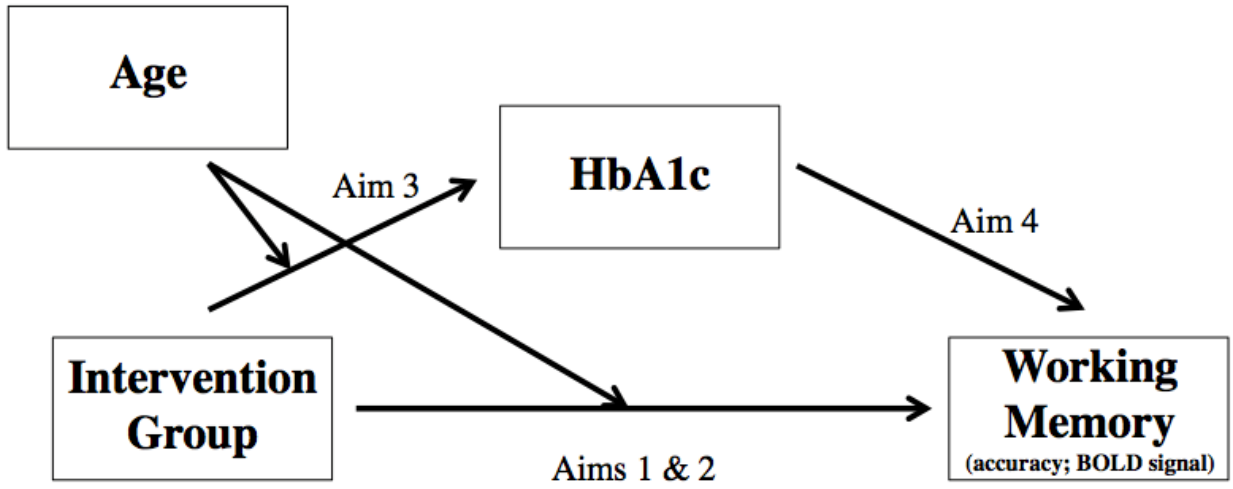


Figure 1: Moderated mediation design: Aims outlined in a moderated mediation design. Aims 1 and 2 examine the direct effect of intervention group on N-back accuracy and task-evoked BOLD signal. Aim 3 examines the direct effect of intervention group on HbA1c levels. Aim 4 examines the mediating effect of HbA1c on the group effects on N-back accuracy and task-evoked BOLD signal.

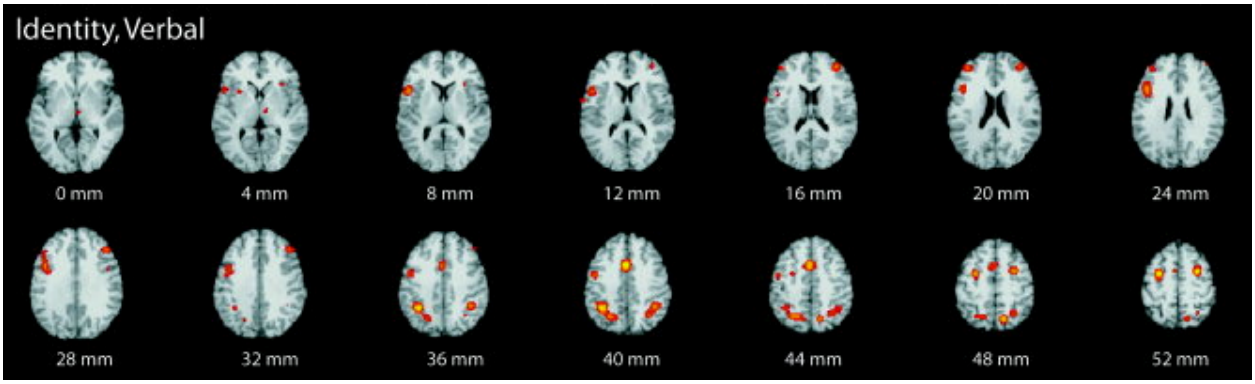


Figure 2: Regional brain activity associated with N-back accuracy: Regions where N-back accuracy is associated with BOLD signal in the Letter N-Back task [77].

2.0 METHODS

2.1 PARTICIPANTS

Participants were recruited from the Action for Health in Diabetes (Look AHEAD) trial. The Look AHEAD trial recruited Type 2 Diabetics between 45−76 years of age. All individuals were eligible if they had a BMI >25kg/m2, AbA1c less than 11% (97 mmol/mol), systolic blood pressure less than 160 mmHg, diastolic blood pressure less than 100 mmHg, and triglycerides less than 600 mg/dL (see The Look AHEAD Research Group, 2003 for full inclusion criteria).

The current study was an ancillary of the Look AHEAD parent trial, titled the Brain MRI study. In the Look AHEAD Brain MRI study, participants from three (Brown University, University of Pennsylvania, University of Pittsburgh) of the 16 Look AHEAD centers were recruited to participate in cognitive and MRI testing at their 10-year follow up. Participants were eligible if they were currently active in the study, had no lapse in follow-up attendance, and passed MRI screening. Participants were eligible to enter the MRI environment if they weighed less than 400 lbs, reported no history of claustrophobia, did not have any surgical metal implants that were not listed as MR safe, had never been exposed to welding or had an x-ray confirming no presence of metal in their body. If the participant was uncertain about claustrophobia or the MRI procedure, a mock MRI scan was provided where participants were shown a full-scale model of the machine, asked to lie in the mock machine and listen to recordings of the sounds that each sequence of the scanner emits. All participants signed an approved informed consent for participation in the MRI study. Participants were remunerated $100.00 for the MRI portion of the study.

A total of 530 participants were eligible for the Brain MRI study and consented across all

three sites. Of the 530 participants, 321 were scheduled and scanned within the designated 2 week window from their 10-year follow-up. Of these, 12 participants did not have complete MRI data during the N-back task and 9 did not have complete behavioral data during the task (recorded response time and accuracy), 9 were excluded due to data collection error or excessive motion during the cognitive task (>2 mm), and 19 were excluded due to poor data quality (unable to register to standard space). Finally, 35 participants were excluded for poor performance on the working memory task (accuracy $< 50\%$). A total of 237 participants were included in the final analyses Figure 3.

2.2 INTERVENTION

The Action for Health in Diabetes (Look AHEAD) Study originated as a lifestyle intervention program for adults with Type 2 Diabetes Mellitus. The main goal of the intervention was to promote healthy eating habits and to increase physical activity in participants while improving their cardiovascular health and lowering mortality rates. Participants were recruited at 16 clinical centers nationwide, totaling over 5,000 participants. Within each site, participants were randomized into two groups: Intensive Lifestyle Intervention (ILI) and Diabetes Support and Education (DSE).

The experimental group, ILI, was given nutrition and exercise plans, as well as professional support and council, with the aim to lose 7-10% of their body weight in the first year of the intervention. Participants in the ILI group were assigned to a calorie restriction diet of 1,200−1,800 total calories containing less than 30% of total calories from fat, of which <10% from saturated fat, and >15% total calories from protein. Participants were also given a physical activity regimen of >175 minutes/week of physical activity such as brisk walking. Physical activity sessions were not monitored, but conducted by each participant at his or her discretion. Participants were encouraged to increase their physical activity by meeting increasing goals. ILI participants participated in weekly meetings for the first six months of the intervention, followed by three meetings per month for the remaining six months of the first year. These meetings involved both one-on-one meetings with researchers as well as

group sessions with other ILI participants. For years two through four, individual meetings were held once per month, as well as monthly contacts by phone or email and information to participate in group classes. Following year four, monthly individual meetings and one annual event were the only forms of contact.

The control group, DSE, was also given professional support, as well as seminars on managing diabetes through diet and exercise, but on a less frequent basis. These participants were given access to three group educational meetings each year. Full information about the groups can be found at [95].

2.3 DESIGN

2.3.1 Weight and HbA1c

Physical assessments were obtained by certified staff blinded to the participants' intervention group. Weight and height were measured in duplicate using a digital scale and stadiometer. HbA1c blood work was completed after at least a 12-h fast and was analyzed by the Central Biochemistry Laboratory (Northwest Lipid Research Laboratories, University of Washington, Seattle, WA) using standardized laboratory procedures. These methods have been previously reported [63, 95].

2.3.2 N-Back Task

Working memory was measured using a standard letter N-back task, containing 1-back and 2-back conditions. The stimuli were arranged in a fixed block design, such that each participant viewed the same block sequence displaying the 1-back and 2-back three times each. The sequence of letters within each block were randomized and unique to each participant and each block. Blocks were 64000ms in length, with each letter being presented for 2000ms. Each block was preceded by a 2000ms instruction screen identifying if the block would be 1-back or 2-back condition and followed by a 2400ms fixation screen. All stimuli were presented on a black screen and printed in white lowercase Arial font in the center of the screen. The

entire task lasted approximately 9 minutes Figure 4. All responses were collected and scored for response time and accuracy.

2.3.3 Protocol

Upon arrival, participants entered a practice room with the researcher containing a desk, two chairs, and a Dell computer equipped with the stimulus presentation software EPrime. Using EPrime, participants completed a practice session of a letter N-Back task, containing 1-back and 2-back conditions. In the 1-back condition, participants were instructed to identify whether the letter currently on the screen matched the letter immediately preceding it. If the current letter matched the previous letter, participants were instructed to press the "m" key on the keyboard using their right index finger. If the current letter did not match the previous letter, participants were instructed to press the "z" key on the keyboard using their left index finger. Participants were provided automatic feedback after each trial, indicating if their response was correct or incorrect. This procedure was repeated for the 2-back task, where participants were instructed to identify if the letter currently on the screen matched the letter presented two letters previously Figure 3. Participants were instructed that the task required constant updating of the letter sequence and that they needed to respond to each letter as quickly and as accurately as possible. Participants were allowed to repeat this practice with feedback up to 5 times, or until they felt comfortable that they understood the task. There was no incidence of a participant failing to complete the practice or displaying inability to perform the 1-back task, at minimum. After the practice session, participants met with the MRI technician to remove all metal and completed an MRI safety form and release, confirming that they were eligible to enter the MRI environment.

2.3.4 MRI Acquisition

Participants were scanned on a Siemens Trio 3-Tesla scanner at Brown University and University of Pennsylvania sites. At the University of Pittsburgh site, participants were scanned on a Siemens Verio 3-Tesla scanner. Despite slightly different models, all scans utilized a 32-channel head coil and the same scan sequence parameters. Additionally, monthly phantom

scans were acquired to confirm that all three sites were collecting accurate and comparable data.

A total of 9 sequences were collected, beginning with a 3 plane localizer and followed by a 3D T1-weighted magnetization prepared rapid gradient echo (MPRAGE) image (sagittal plane, TR=1900, TE=2.89, FOV=250mm, flip angle = 9°) consisting of 176 slices generating 1mm × 1mm × 1mm voxels. Additional structural sequences (3D T2 Fluid Attenuated Inversion Recovery FLAIR and 3D T2-weighted Fast Spin Echo FSE) and one functional resting state sequences (Blood Oxygen Level Dependent BOLD) were collected before the working memory BOLD sequence. The n-back working memory sequence (TR=2000, TE=28, FOV=224, flip angle = 75°) was collected in the axial plane at a resolution of $3.2 \times 3.2 \times 3.2$ mm voxels across 36 slices.

The N-back task was administered using the software Eprime 2.0 (Psychology Software Tools), displayed on a screen viewable to the participant through a tilted mirror attached to the head coil. To ensure that the participants remembered the task and could perform it in the scanner, they were instructed to complete a practice run of 1 block each of the 1-back and 2-back with feedback ("correct" or "incorrect" displayed on the screen after each response) immediately before initiating the scanner sequences. Participants were given two response gloves, one for each hand, with buttons located under each finger and thumbs. They were instructed to respond to the tasks using their index fingers only; if they wanted to respond "match" they were to press down with their right index finger, and if they wanted to respond "not a match" they were to press down with their left index finger. These were the same fingers used in the out-of-scanner practice. All responses and response times were collected and output into text files in Eprime.

2.4 STUDY 1: GROUP EFFECTS OF WORKING MEMORY

2.4.1 fMRI analyses

MPRAGE and N-Back EPI sequences from all three sites were made available on a cloud based server and downloaded onto a single processing computer. Images were concatenated into nifti files from their original dicom format. Using Functional Magnetic Resonance Imaging of the Brain (FMRIB) Analysis Group, Oxford University, UK Software Library (FSL) all images were processed using standard techniques of brain extraction (skull stripping) and motion correction. First level analyses were then applied to each individual participant in data space using FEAT in FSL. A GLM using multiple regression was created to generate a design matrix to isolate signal during each task (1-back, 2-back) and averaged across blocks. From this, four contrasts were generated, illustrating voxel-wise statistical maps of activation during: 1-back > baseline, 2-back > baseline, 1-back > 2-back, and 2-back > 1-back.

MPRAGE images for each individual were registered into standard MNI 152 space using FSL's linear registration algorithm, FLIRT [50]. For unknown reasons, 31 participants' MPRAGE images flipped 180 degrees along the y-axis during registration. To reverse this, the registration technique was altered to constrain the algorithm's search of best fit to the x and z axes. This alleviated the registration error in 12 of the participants. The remaining 19 were excluded from the current analyses Figure 3.

Higher-level analyses were conducted on the group level for the remaining 237 participants using FSL FEAT. First, each of the four contrasts were created comparing intervention groups (see Figure 5a) to determine group effects of activation during the N-back task. Next, accuracy and response time during the 1-back and 2-back tasks were added to the multiple regression model in order to identify if behavior during the task predicted regional activity. This model was run separately for ILI and DSE participants so that voxel-wise statistical maps could be compared between groups (see Figure 5b). Finally, an interaction term was generated by multiplying intervention group (labeled 1 – 2) × age. Age, intervention group, and the interaction term of group × age was modeled to generate a voxel-wise analysis of regions where the effect of group on activation was moderated by age (see Figure 5c). Site

(Philadelphia, Pittsburgh, Providence) was included in every model discussed and orthogonalized to account for and remove any variability in signal due to location of scan. All group-level voxel-wise analyses were conducted using a cluster threshold of $z > 2.3$ and $p < 0.05$.

All group-level voxel-wise analyses were conducted using a cluster threshold of $z > 2.3$ and $p < 0.05$. Regions of interest were then identified based on the functionally defined significant clusters. The FSL function featquery was then used to extract an average percent signal change and z-score for each cluster for each individual.

2.4.2 Statistical analyses

All demographic variables of interest (age, gender, HbA1c, weight, BMI, diabetes duration) were compared between groups using independent samples t-tests, to ensure quality of randomization in the subsample used and to identify any group effects in year 10. Independent samples t-tests were also conducted with all N-back behavioral measures (response time and accuracy for the 1-back and 2-back tasks) to determine group differences in working memory performance.

Correlations were run to determine the relationship between demographic variables (age, gender, weight, BMI, diabetes duration) and N-back behavioral measures (response time and accuracy for the 1-back and 2-back tasks). To determine if site contributed to any variance in N-back performance, a One-Way ANOVA was conducted with an LSD post-hoc analysis to identify differences between all three sites in accuracy and response times for the 1-back and 2-back tasks.

Intervention group was then entered into a linear regression model to determine if group predicted N-back performance (Figure 6a). To examine any relationship between group, age, and N-back performance, a multiple regression model was created using age as a moderator (Figure 6b). An interaction term of group $\times$ age was calculated and entered in a stepwise fashion. Gender was added to the model as a covariate, so that the regression consisted of three models: 1) gender; 2) gender, age, group; 3) gender, age, group, age $\times$ group.

2.5 STUDY 2: RELATIONSHIP BETWEEN GROUP, HBA1C, AND WORKING MEMORY

2.5.1 fMRI analyses

Image processing and first level analyses were conducted as described above in section 2.4.1. HbA1c levels at year 10 were only available for 163 of the 237 participants previously described.

Higher-level analyses were conducted on the group level for the 163 participants using FSL FEAT. First, HbA1c was entered into the group level model to identify regions in each of the N-back contrasts (1-back, 2-back, 1>2-back, 2>1-back) where activity was correlated with HbA1c levels. Then, to examine age as a moderator of the effect of HbA1c on N-back evoked brain activity, an interaction term of age × HbA1c was entered into the model with age, HbA1c, and a variable for the intercept consisting of a value of 1 for each subject. This was modeled to show both regions where age moderates the relationship between HbA1c and higher activity, and lower activity. Site (Philadelphia, Pittsburgh, Providence) was included in every model discussed and orthogonalized to account for and remove any variability in signal due to location of scan.

All group-level voxel-wise analyses were conducted using a cluster threshold of $z > 2.3$ and $p < 0.05$. Regions of interest were then identified based on the functionally defined significant clusters. The FSL function featquery was then used to extract an average percent signal change and z-score for each cluster for each individual. Values from the clusters identified in the previous study as significantly correlated with N-back performance were then entered into a moderated mediation model with HbA1c to determine if HbA1c mediates the relationship between intervention group and brain activity in each cluster as a function of age (Figure 7).

2.5.2 Statistical analyses

All demographic variables of interest (age, gender, HbA1c, weight, BMI, diabetes duration) were compared between groups using independent samples t-tests, to ensure quality of ran-

domization in the subsample used and to identify any group effects in year 10. Independent samples t-tests were also conducted with all N-back behavioral measures (response time and accuracy for the 1-back and 2-back tasks) to determine group differences in working memory performance.

Correlations were run to determine the relationship between demographic variables (age, gender, weight, BMI, diabetes duration) and N-back behavioral measures (response time and accuracy for the 1-back and 2-back tasks). To determine if site contributed to any variance in N-back performance, a One-Way ANOVA was conducted with an LSD post-hoc analysis to identify differences between all three sites in accuracy and response times for the 1-back and 2-back tasks.

To examine any relationship between group, age, and N-back performance, a multiple regression model was created using age as a moderator. An interaction term of group $\times$ age was calculated and entered in a stepwise fashion. Gender was added to the model as a covariate, so that the regression consisted of three models: 1) gender; 2) gender, age, group; 3) gender, age, group, age $\times$ group.

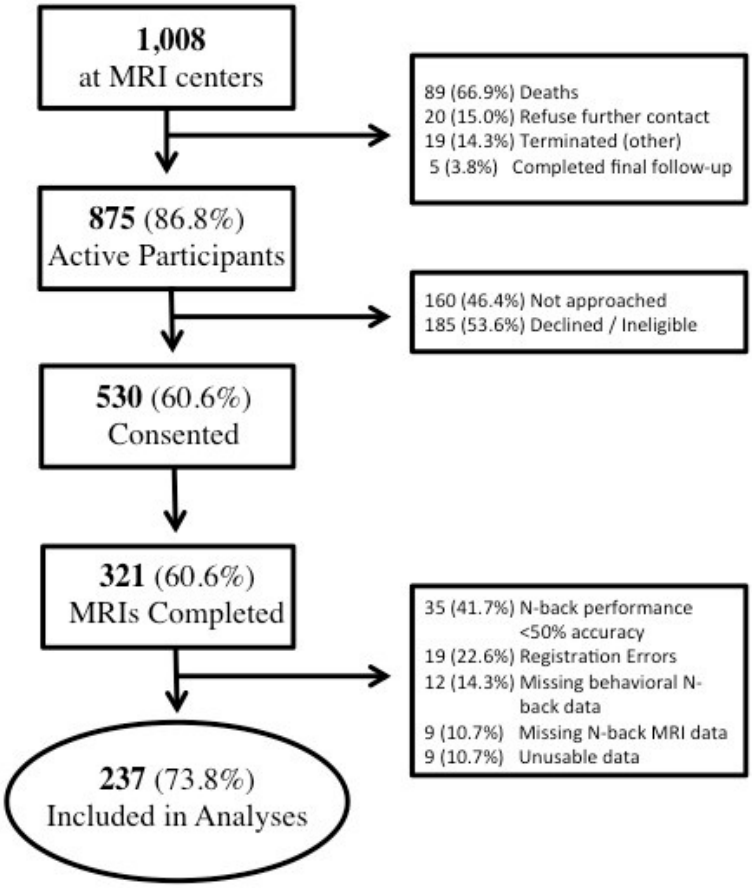


Figure 3: LookAHEAD brain ancillary study flow diagram: Subject pool with drop out information combined for three sites, Philadelphia, Pittsburgh, Providence.

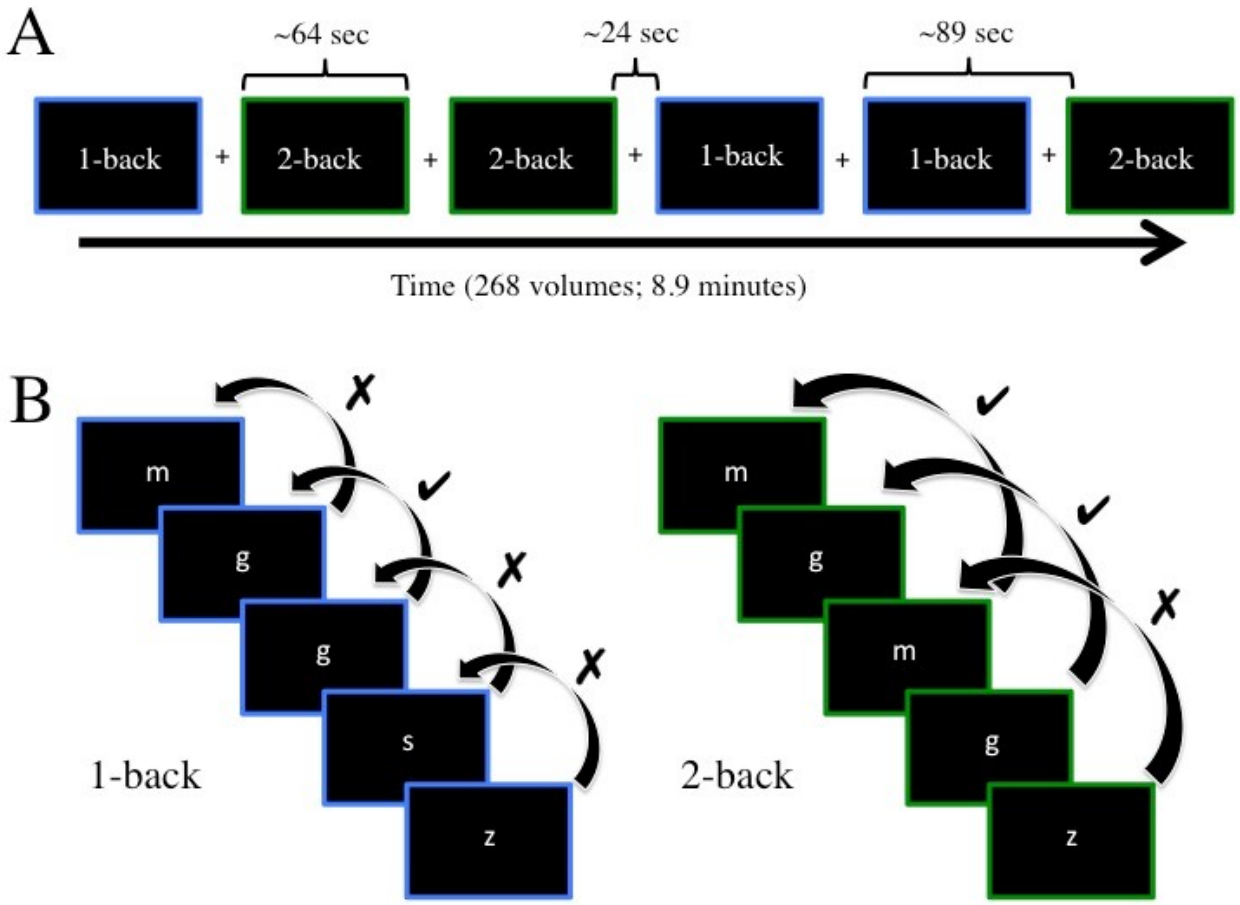


Figure 4: N-back stimuli design: A) fMRI block design in fixed order for three presentations of 1-back and 2-back conditions, separated by 24sec fixation. B) 1-back and 2-back letter stimuli presentation sequence. ✓ indicates correct match trial; ✗ indicates non-match trial.

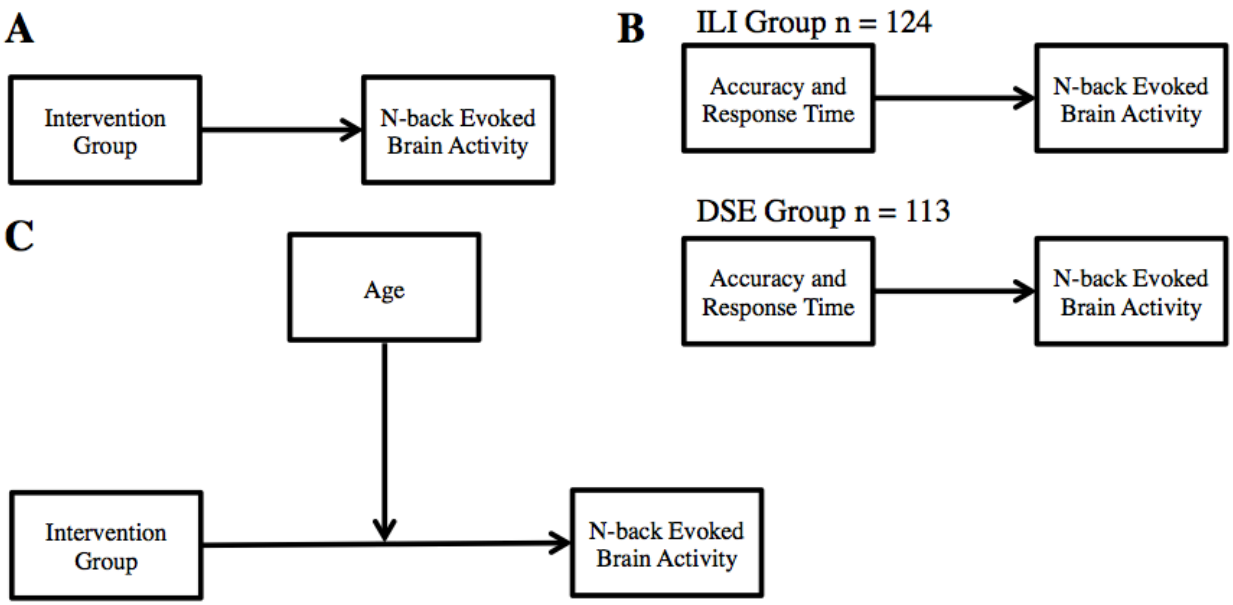


Figure 5: Statistical models for fMRI analyses

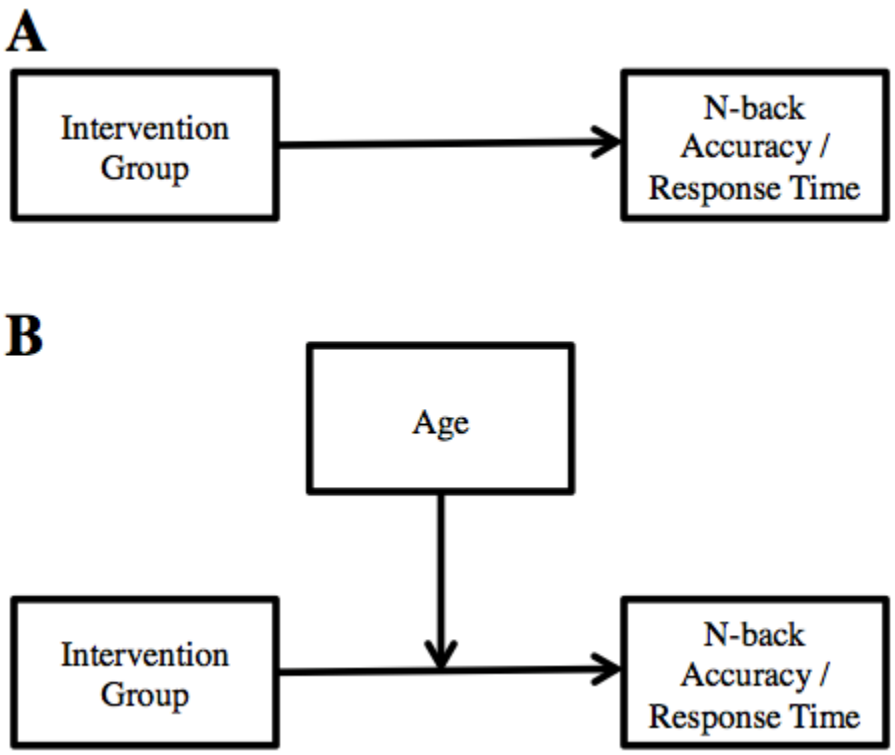


Figure 6: Moderation model of age on relationship between group and N-back performance: A) Predicting N-back performance by intervention group. B) Predicting N-back performance by intervention group as a function of age.

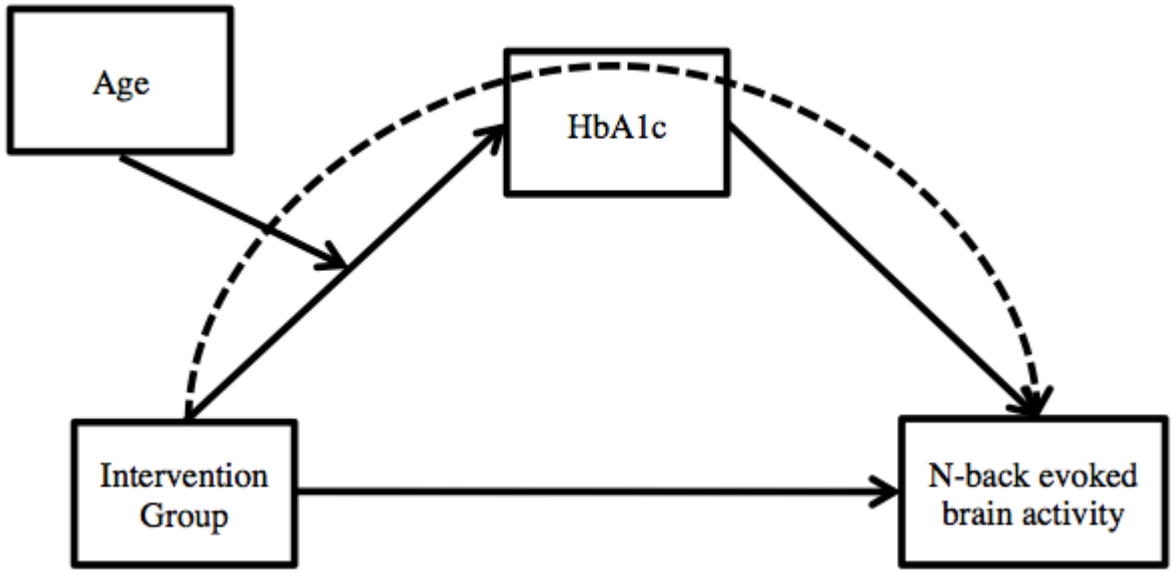


Figure 7: Moderated mediation for fMRI analyses: Solid lines indicate direct relationships tested. Dotted line indicates the indirect effect of intervention group on N-back brain activity as mediated by HbA1c.

3.0 RESULTS

There were no significant differences between the ILI and DSE groups in gender, age, diabetes duration, baseline HbA1c, or baseline weight in this subsample of the Look AHEAD participant pool. There was a significant difference between groups in baseline BMI, such that the DSE group ($M = 36.103$, $SD = 5.856$) had a higher starting BMI than the ILI group ($M = 34.442$, $SD = 5.149$) ($t(235) = -2.356$, $p = 0.019$).

At 10 years after the start of the intervention, there was a significant difference between groups in BMI, such that the DSE group ($M = 34.352$, $SD = 5.808$) had a significantly higher average BMI than the ILI group ($M = 32.050$, $SD = 4.935$) ($t(198) = -3.018$, $p = 0.003$). There were no significant differences between groups on HbA1c or weight at year 10 (see Table 1 for all means, standard deviations, and t-test results).

3.1 BEHAVIORAL N-BACK RESULTS BY SITE

There are several significant differences in N-Back performance between sites (Table 2). On 1-back response time, participants at Providence ($M = 898.17$, $SD = 203.78$) were significantly faster than participants at Philadelphia (M = 1074.86, SD = 228.56) and Pittsburgh ($M = 1047.74$, $SD = 198.38$) sites ($F(2, 236) = 15.56$, $p = 0.00$). There was no significant difference between response time on the 1-back task between participants at Penn and Pitt. There was no significant difference between sites on 1-back accuracy ($F(2, 236) = 0.791$, $p = 0.455$).

On 2-back response time, participants at Providence ($M = 1141.15$, $SD = 226.25$) were significantly faster than participants at Philadelphia ($M = 1349.20$, $SD = 253.54$) and

Table 1: Demographic information

	Group 1 – ILI (n = 124)			*Group 2 – DSE (n = 113)*			*Group Differences*	
	n	Mean (SD)	Range	n	Mean (SD)	Range	t	p
Age	124	68.32 (6.62)	55 - 84	113	66.81 (5.99)	54 - 80	1.87	0.06
Gender (% female)	124	65.1%		113	72.9%		-1.31	0.19
Baseline Weight (kg)	124	95.80 (16.14)	62.30 – 131.50	113	99.24 (17.96)	60.65 – 167.50	-1.21	0.26
Year 10 Weight (kg)	118	89.38 (15.88)	57.80 – 138.20	113	92.96 (17.65)	58.80 134.50	-1.64	0.10
Baseline HbA1c	124	7.19 (1.13)	4.70 – 11.00	113	7.41 (1.42)	5.20 – 14.50	-1.32	0.19
Year 10 HbA1c	82	7.25 (1.50)	5.1 – 13.3	87	7.52 (1.55)	5.4 – 13.7	-1.16	0.25
Diabetes Duration	124	6.37 (7.36)	0.00 – 51.00	113	5.92 (5.89)	0.00 – 30.00	0.52	0.61
Baseline BMI	124	34.44 (5.15)	25.17 – 47.27	113	36.10 (5.86)	26.02 – 60.06	-2.36	0.02*
Year 10 BMI	99	32.05 (4.93)	22.95 – 47.56	101	34.35 (5.81)	23.89 – 49.81	-3.02	0.003**
1-Back Reaction Time	124	990.46 (208.04)	479.34 – 1857.72	113	1034.31 (235.31)	574.57 – 1667.37	-1.54	0.12
1-Back Accuracy	124	0.93 (0.10)	0.60 – 1.00	113	0.91 (0.10)	0.51 – 1.00	1.18	0.24
2-Back Reaction Time	124	1258.87 (250.89)	743.63 – 2149.48	113	1266.17 (257.23)	635.79 – 2073.81	-0.22	0.82
2-Back Accuracy	124	0.81 (0.10)	0.55 – 1.00	113	0.82 (0.12)	0.55 – 1.00	-0.68	0.50

* $p < .05$

** $p < .01$

Pittsburgh ($M = 1284.79$, $SD = 239.34$) sites ($F(2, 236) = 14.796$, $p = 0.00$). There was no significant difference between response time on the 2-back task between participants at Penn and Pitt.

There was no significant total effect of site on 2-back accuracy ($F(2, 243) = 2.839$, $p = 0.06$); however, participants at Philadelphia ($M = 0.796$, $SD = 0.105$) were significantly less accurate than participants at Providence ($M = 0.839$, $SD = 0.106$) ($p = 0.028$). There was no significant difference in accuracy between Providence or Philadelphia sites and Pittsburgh ($M = 0.805$, $SD = 0.120$).

3.2 STUDY 1: GROUP EFFECTS OF WORKING MEMORY

3.2.1 N-Back behavioral results

Across all participants and controlling for site, a Pearson's correlation reveals age is significantly correlated with 1-back response time ($r = 0.266$, $p = 0.000$), 1-back accuracy ($r = -0.241$, $p = 0.002$), 2-back response time ($r = 0.234$, $p = 0.002$), and $r = -0.312$,

Table 2: N-back performance by site

		Mean (SD)	T-tests *(p)*		
			Philadelphia	Pittsburgh	Providence
Philadelphia	*1-Back Reaction Time*	1074.86 (228.56)	0		
	1-Back Accuracy	0.913 (0.106)	0		
	2-Back Reaction Time	1349.20 (253.54)	0		
	2-Back Accuracy	0.799 (0.105)	0		
Pittsburgh	*1-Back Reaction Time*	1047.47 (198.38)	0.401	0	
	1-Back Accuracy	0.920 (0.107)	0.622	0	
	2-Back Reaction Time	1284.79 (239.34)	0.082	0	
	2-Back Accuracy	0.805 (0.120)	0.709	0	
Providence	*1-Back Reaction Time*	898.17 (203.78)	0.000**	0.000**	0
	1-Back Accuracy	0.933 (0.088)	0.214	0.417	0
	2-Back Reaction Time	1141.15 (226.26)	0.000**	0.000**	0
	2-Back Accuracy	0.839 (0.106)	0.028*	0.054	0

* $p < .05$

** $p < .01$

$p = 0.000$), such that older age is associated with lower accuracy and slower response time. Weight at year 10 is significantly correlated with 1-back response time ($r = -0.217$, $p = 0.005$), 2-back response time ($r = -0.242$, $p = 0.002$), and 2-back accuracy ($r = 0.152$, $p = 0.050$), such that higher weight is associated with faster response time on both tasks and better accuracy on the 2-back task, but not the 1-back task (see Table 3).

Within only ILI participants and controlling for site, age is significantly correlated with 1-back response time ($r = 0.224$, $p = 0.044$), 2-back response time ($r = 0.224$, $p = 0.044$), and 2-back accuracy ($r = -0.463$, $p = 0.000$), such that older age is associated with slower response time and lower accuracy. Age was not significantly associated with 1-back accuracy. Weight ($r = 0.253$, $p = 0.022$) and BMI ($r = 0.273$, $p = 0.014$) at year 10 are significantly correlated with 2-back accuracy only, such that higher weight and BMI are associated with better accuracy.

Within only DSE participants and controlling for site, age was significantly correlated with 1-back response time ($r = 0.367$, $p = 0.001$), 1-back accuracy ($r = -0.310$, $p = 0.004$), and 2-back response time ($r = 0.273$, $p = 0.011$), such that older age is associated with slower

response time in the 1-back and 2 back tasks, and worse accuracy in the 1-back task only. Gender was significantly associated with 2-back accuracy ($r = -0.227$, $p = 0.036$) such that males had higher accuracy than females. This is also seen in an independent samples t-test where males ($M = 0.858$, $SD = 0.093$) had significantly better accuracy on the 2-back task than females ($M = 0.803$, $SD = 0.126$) ($t(116) = 2.234$, $p = 0.027$). Diabetes duration was significantly correlated with 1-back accuracy ($r = -0.246$, $p = 0.022$) and marginally with 2-back accuracy ($r = -0.029$, $p = 0.054$), such that longer diabetes duration is associated with poorer accuracy. Weight at year 10 was significantly correlated with 1-back response time ($r = -0.289$, $p = 0.007$) and 2-back response time ($r = -0.351$, $p = 0.001$), such that higher weight was associated with faster response time. BMI, nor HbA1c, at year 10 were significantly associated with any measures of the N-back task.

Despite differences within groups, use of a multiple linear regression determined that intervention group did not significantly predict 1-back response time ($\beta = -0.102$, $p = 0.087$), 1-back accuracy ($\beta = 0.099$, $p = .111$), 2-back response time ($\beta = -0.008$, $p = 0.886$), or 2-back accuracy ($\beta = -0.023$, $p = 0.708$).

Hierarchical linear regression was used to examine age as a moderator of the relationship between intervention group and N-back performance. Gender and site were included in the model as covariates, accounting for 2.6% of the variance in 2-back accuracy. The interaction term of group x age confirmed that age is a significant moderator of the relationship between group and 2-back accuracy ($\beta = -1.126$, $p = 0.049$), accounting for 12.3% of the variability in 2-back accuracy (see Table 4). There were significant main effects of age ($\beta = -0.301$, $p = 0.000$), such that older age predicted lower accuracy. There was no significant main effect of intervention group. Age was not a significant moderator in the 1-back response time, 1-back accuracy, or 2-back response time measures.

Because age and diabetes duration were significantly correlated and to confirm that diabetes duration was not driving the age $\times$ group interaction, diabetes duration was added to model 1 as a covariate with site and gender. Adding diabetes duration as a covariate strengthened the model, as the age $\times$ group interaction accounted for 14.3% of the variability in 2-back accuracy; an additional 2.0% from the previous model.

To further examine the relationship of the moderation and to determine direction, the

data was graphed in two ways. First, age was graphed against 2-back accuracy for both groups (Figure 8). The slope of regression line in the ILI group is significant in the model and displays that older adults in the ILI group displayed poorer accuracy, while the slope of the DSE group is not significant.

Second, age was converted into two groups using a median split at age of 67 years (Figure 9). T-test were conducted to compare the four groups, demonstrating that in the ILI group the adults >67 years ($M = 0.778$, $SD = 0.1.4$) had significantly lower accuracy compared to the adults <67 years ($M = 0.850$, $SD = 0.088$) ($t(124) = 4.035$, $p = 0.000$). There is no significant difference in the DSE group between adults >67 years ($M = 0.803$, $SD = 0.123$) and adults <67 years ($M = 0.836$, $SD = 0.116$) ($t(113) = 1.478$, $p = 0.142$). When comparing age groups between randomization groups, there is no significant difference between adults <67 in the ILI group and the DSE group ($t(104) = 0.673$, $p = 0.502$), nor was there a significant difference between adults >67 in the ILI and DSE groups ($t(136) = -1.298$, $p = 0.196$). Therefore, the >67 adults in the ILI group are driving the age × group interaction.

3.2.2 fMRI results

Group level analyses identified regions in the occipital cortex that were significantly correlated with the location of the scan (Philadelphia, Pittsburgh, Providence) (Figure 10). These regions were only correlated with site in the 2-back > 1-back contrast, however confirm site as a necessary covariate in all analyses.

Several regions survived cluster thresholding ($z > 2.3$, $p < 0.05$) and were identified in the group level analysis where brain activity (BOLD signal) was correlated with behavioral N-back measures across all participants. Higher activity in the 1-back > 2-back contrast was associated with 1-back response time in two significant clusters located in the right middle frontal gyrus and right lateral occipital and precuneus regions (Figure 11a and Table 5). Accuracy on the 1-back task was associated with a significant cluster in the right insula, such that better accuracy on the 1-back resulted in more activity in the 1-back > 2-back contrast (Figure 11a and Table 5). Higher activity in the 2-back > 1-back contrast was associated

with 2-back response time in a region of the right middle frontal gyrus (Figure 11b and Table 5). 2-back accuracy was significantly associated with activity in the lateral occipital gyrus and frontal pole and precuneus during the 2-back > 1-back contrast (Figure 11b and Table 5).

When comparing intervention group and activity during the 1-back > 2-back and 2-back > 1-back contrasts, no regions met cluster threshold for significance. Therefore, there was no significant relationship between intervention group and N-back brain activity in this sample. However, when separating groups, overlapping regions of activation were revealed. Within the ILI group, regions in the right inferior frontal gyrus, right frontal pole, right superior temporal gyrus, and right middle temporal gyrus were associated with activity during the 1-back > 2-back contrast and 1-back response time (Figure 12 and Table 6). There were no regions that reached cluster threshold in the DSE group in relation to 1-back response time.

There were no regions that reached cluster threshold in the ILI group that were associated with activity during the 1-back > 2-back contrast and 1-back accuracy; however, one cluster in the DSE group met threshold in the right insula, covering the supramarginal gyrus and parietal operculum cortex (Figure 12).

Comparing activity in the 2-back > 1-back contrast between groups in relation to 2-back performance, there ILI group showed regions in the right inferior frontal gyrus, right insula, and right frontal pole were associated with activity and 1-back response time (Figure 12). There were no regions that reached cluster threshold in the DSE group in relation to 2-back response time.

In the ILI group, regions in the left lateral occipital cortex were associated with 2-back accuracy and activity during the 2-back > 1-back contrast. However, in the DSE group, regions in the right insula and right parietal operculum cortex were associated with 2-back accuracy (Figure 12). Despite differences in significant clusters associated with N-back performance, there remains no significant effect of group on behavior-correlated activity.

Clusters identified in both ILI and DSE groups were large and covered multiple anatomical brain regions. For this reason, voxels with peak BOLD signal (z-score) were identified within each cluster and reported in Table 6.

The interaction term age × group was generated and entered into a group level analysis

to determine if the relationship between group and activity was moderated by age. No regions reached cluster threshold for the main effect of group, main effect of age, nor the interaction term.

Z-scores of average activity for each individual was extracted for each of the reported clusters associated with N-back performance and brain activity during the N-back task (reported in Table 6). The average z-scores for each region was then entered into a moderation model to determine if age moderated any effect of group on activation in that region. Age did not moderate the relationship between group and activity in any of the regions, nor were there any main effects of group or age. For visualization purposes, graphs of each region can be found in Figure 13.

3.3 STUDY 2: HBA1C MEDIATES GROUP EFFECTS OF WORKING MEMORY

3.3.1 N-back behavioral results

As previously reported, HbA1c at year 10 was not significantly different between ILI and DSE groups, nor was it significantly correlated with N-back performance.

Hierarchical linear regression including gender and site as covariates was used to examine age as a moderator of the relationship between HbA1c levels and N-back accuracy (Table 7). There were no main effects of age or HbA1c in 1-back or 2-back response times, nor was age a moderator for either of these variables.

Examining 1-back accuracy, there was a significant main effect of age ($\beta = -0.265$, $p = 0.001$), such that older age predicted lower 1-back accuracy. There was no main effect of HbA1c. The interaction term of age $\times$ HbA1c identified that age is a significant moderator of the relationship of HbA1c and 1-back accuracy ($\beta = 1.470$, $p = 0.026$), such those with high HbA1c levels and were <67 years old performed better than those with high HbA1c levels and were >67 years old (see Figure 14a and Figure 15a).

Examining 2-back Accuracy, there was a significant main effect of age ($\beta = -0.327$,

$p = 0.000$), such that older age was associated with lower 2-back accuracy. There was no significant main effect of HbA1c. The interaction term of age × HbA1c identified that age is a significant moderator of the relationship between HbA1c and 2-back accuracy ($\beta = 1.494$, $p = 0.000$), such those with high HbA1c levels and were <67 years old performed better than those with high HbA1c levels and were >67 years old (see Figure 14b and Figure 15b).

A hierarchical linear regression model was then used to determine if age moderated the relationship between randomization group and HbA1c in year 10, maintaining site, gender, and diabetes duration as covariates. There was a significant main effect of age ($\beta = -0.195$, $p = 0.013$), such that older age was associated with lower HbA1c. There was no significant main effect of randomization group, nor did age significantly moderate the relationship between group and HbA1c level at year 10 (Table 8).

Additional post-hoc analyses of group characteristics reveal that there are the individuals >67 years old in the ILI group ($M = 30.71$, $SD = 4.19$) have a significantly lower mean BMI at year 10 than >67 years old in the DSE group ($M = 33.32$, $SD = 5.21$) ($t(99) = -2.771$, $p = 0.007$). There is no significant difference between ILI and DSE groups among individuals <67 years old (Figure 16).

Despite no significant direct effects of randomization group on N-back performance, as reported in Study 1, the indirect effect of HbA1c as a mediator of any relationship between group and N-back performance was tested. HbA1c in year 10 was not a significant mediator of any relationship between randomization group and performance on any N-back measure (1-back response time, 2-back response time, 1-back accuracy, 2-back accuracy).

In sum, age significantly moderated both the relationship between group and N-back performance, such that the older adults in the ILI group performed significantly worse than younger adults in the ILI group, and the relationship between HbA1c on N-back performance, such that older adults with lower HbA1c performed the worst. A summary of tested relationships is depicted in Figure 17.

3.3.2 fMRI results

Higher level analyses identified several regions where brain activity during the N-back task was associated with HbA1c levels in year 10, across all participants. Activity associated with the 1-back > 2-back contrast was associated with HbA1c levels in regions of the right anterior cingulate cortex, right frontal pole, left insula, right caudate, and right lateral inferior frontal cortex (Figure 18a) that met cluster threshold ($z > 2.3$, $p < 0.05$). Four regions were identified by the cluster analysis where activity during the 2-back > 1-back contrast was associated with HbA1c, localized mainly in the left insula and anterior cingulate cortex, but also in the right hippocampus and putamen (Figure 18b).

The cluster analysis yielded large clusters that covered multiple anatomical regions in both analyses. Therefore, coordinates of voxels with peak activation within each cluster was identified for both the 1-back > 2-back contrast (Table 9) and 2-back > 1-back contrast (Table 10).

Breaking participants down into randomization group, a higher-level analysis determined that there were no group differences in regions that correlated with HbA1c levels. Examining just the ILI group, no significant clusters were identified that met threshold in the 1-back > 2-back, nor the 2-back > 1-back contrasts. Similarly, in just the DSE group there were no significant clusters in either contrast.

Mirroring behavioral analyses, age was added to the model and an interaction term of HbA1c × Age was generated to determine if age moderated any relationship between HbA1c and brain activity during the N-back task across all participants. There were no significant clusters where age moderated the relationship between HbA1c and activity during any of the N-back contrasts. To further visualize any relationship age may have on the relationship between HbA1c and brain activity during the N-back, peak voxels from Table 10 were used as regions of interest (ROI). Spheres of ∼10mm diameter (3 voxels) from the peak coordinate were created and average BOLD signal in z-scores from the 2-back > 1-back contrast was calculated for each individual for each ROI. These values were then entered into a moderation model, where age did not significantly moderate the relationship between HbA1c and activity in any region. The regions are identified in Figure 19 and the relationship

between age, HbA1c and activity is graphed in Figure 20. Despite no main effects or a significant moderation by age, the graphs show a trend for the adults <65 year old who have higher HbA1c levels to have higher activity during the 2-back task.

Table 3: Correlations

	1-Back Reaction Time	1-Back Accuracy	2-Back Reaction Time	2-Back Accuracy	Age	Gender	Diabetes Duration	Year 10 BMI	Year 10 Weight (kg)	Year 10 HbA1c
1-Back Reaction Time	1									
1-Back Accuracy	-0.458 0.000**	1								
2-Back Reaction Time	0.623 0.000**	-0.038 0.622	1							
2-Back Accuracy	-0.398 0.000**	0.465 0.000**	-0.258 0.001**	1						
Age	0.266 0.000**	-0.241 0.002**	0.234 0.002**	-0.312 0.000**	1					
Gender	0.064 0.407	-0.127 0.100	0.112 0.147	-0.097 0.211	-0.060 0.440	1				
Diabetes Duration	0.030 0.697	-0.084 0.278	-0.029 0.707	-0.110 0.158	0.074 0.342	0.027 0.725	1			
Year 10 BMI	-0.098 0.206	-0.012 0.879	-0.127 0.100	0.092 0.235	-0.167 0.031*	0.117 0.132	-0.001 0.988	1		
Year 10 Weight (kg)	-0.217 0.005*	0.117 0.132	-0.242 0.002**	0.152 0.050	-0.159 0.039*	-0.335 0.000**	-0.049 0.524	0.796 0.000**	1	
Year 10 HbA1c	0.071 0.360	-0.037 0.634	-0.022 0.774	0.008 0.917	-0.202 0.009**	0.122 0.115	0.018 0.820	0.139 0.073	0.069 0.378	1

* $p < .05$

** $p < .01$

Table 4: Age moderates relationship between group and N-back performance

1-back Accuracy					
Model		β	t	p	R^2
1	Gender	-0.148	-2.333	0.020*	0.020
	Site	0.082	1.294	0.197	
2	Age	-0.308	-5.026	0.000**	0.109
	Randomization	0.095	1.549	0.123	
3	Age x Group	-0.003	-0.005	0.996	0.106
2-back Accuracy					
Model		β	t	p	R^2
1	Gender	-0.121	-1.916	0.056	0.026
	Site	0.142	2.237	0.026*	
2	Age	-0.301	-4.928	0.000**	0.113
	Randomization	-0.029	-0.472	0.638	
3	Age x Group	-1.126	-1.981	0.049*	0.123

* $p < .05$

** $p < .01$

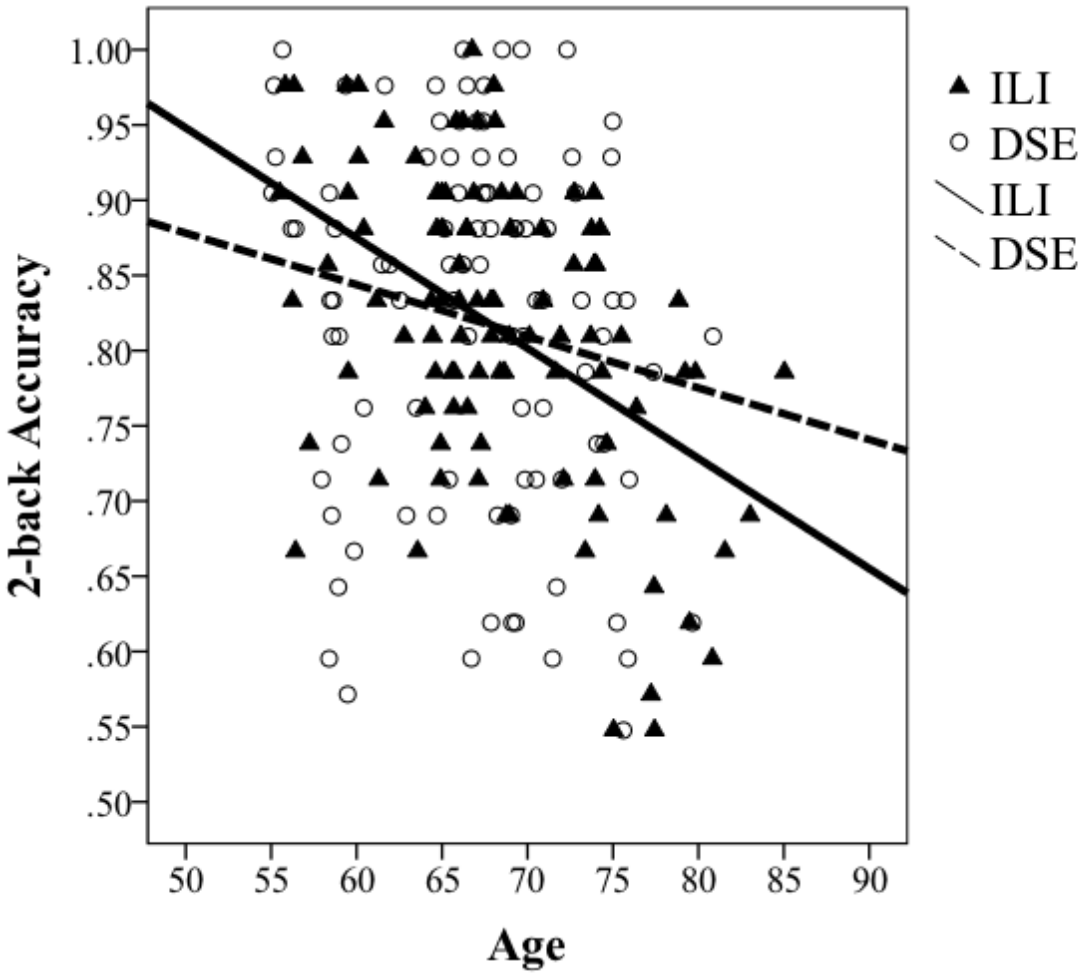
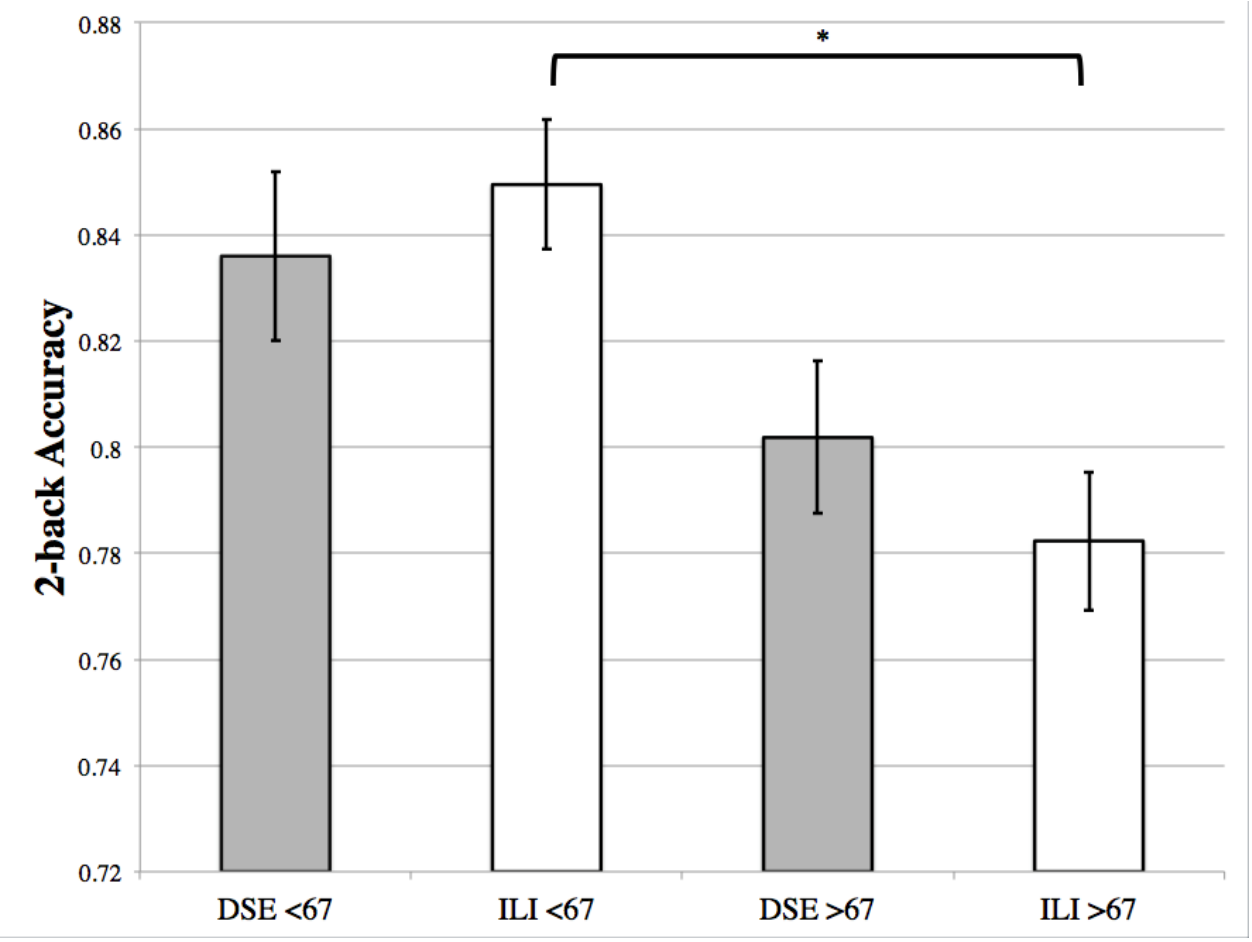


Figure 8: Interaction of age and group in 2-back accuracy



* $p < .01$

Figure 9: Median split of age on 2-back accuracy

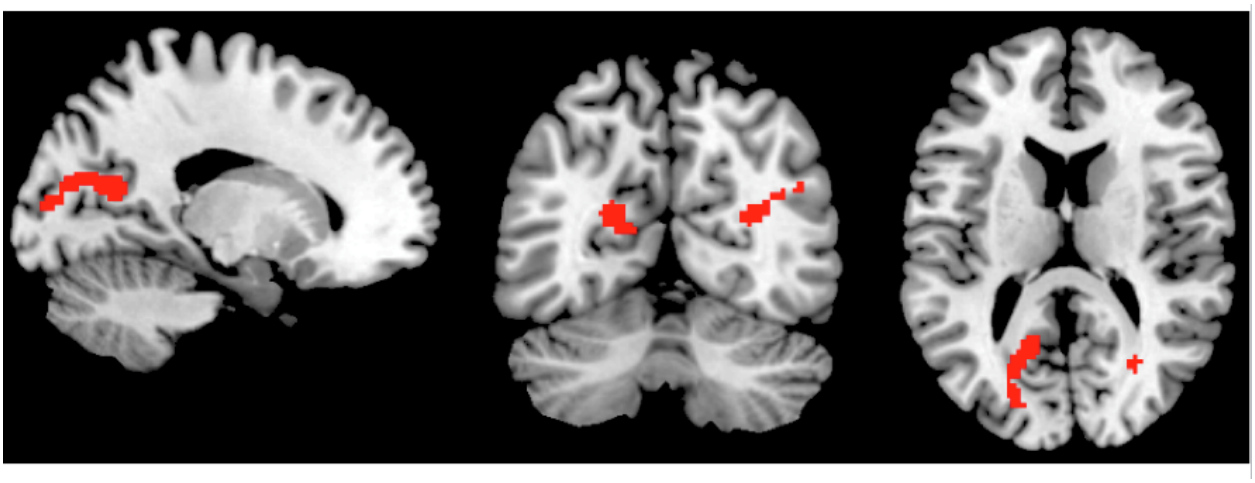


Figure 10: Clusters associated with MRI site and 2-back > 1-back BOLD signal: Cluster threshold $z > 2.3$, $p < .05$.

Table 5: Significant clusters associated with N-back performance

	Number of Voxels	Maximum Z-score	X (mm)	Y (mm)	Z (mm)
2-back > 1-back					
Response time					
r middle frontal gyrus	487	4.34	44	36	30
Accuracy					
l lateral occipital / l inferior temporal	576	3.85	-12	-74	54
l precuneus	443	3.5	-48	-78	8
1-back > 2-back					
Response Time					
r middle frontal gyrus	1507	4.07	44	-60	50
r lateral occipital / precuneus	817	4.71	38	54	20
Accuracy					
r insula	625	3.83	40	-12	14

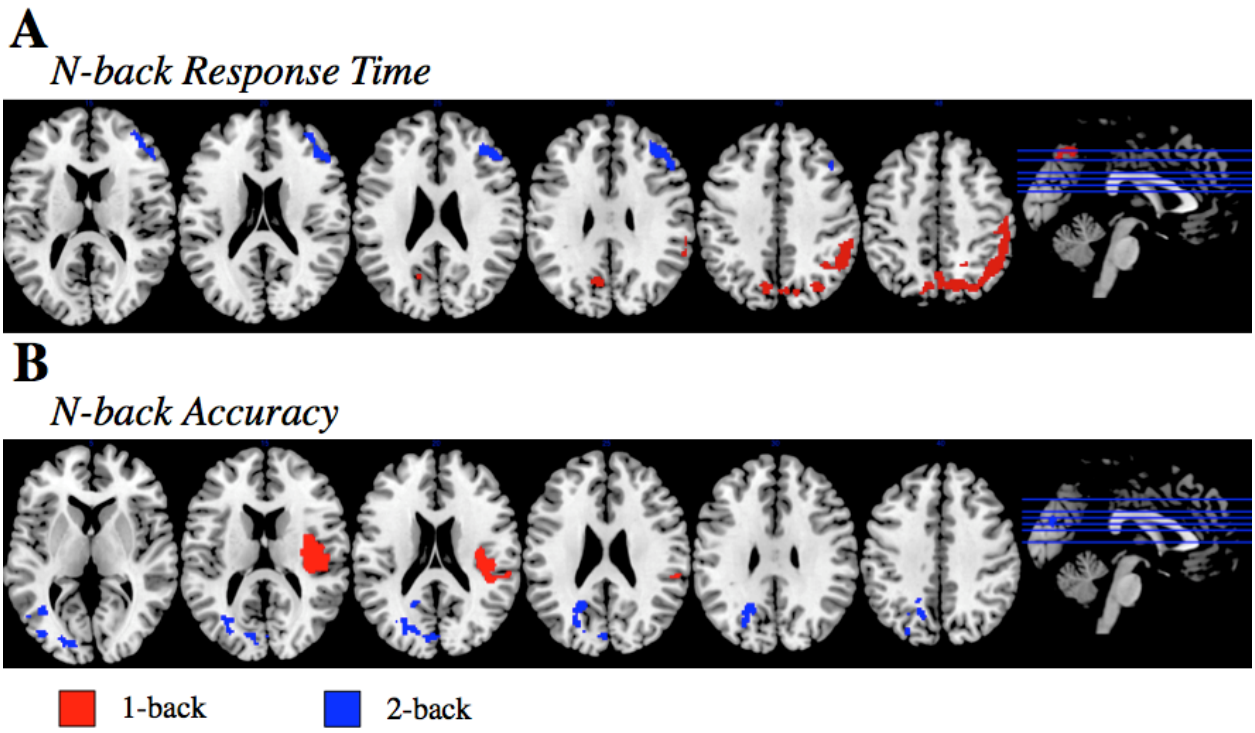


Figure 11: Brain regions associated with N-back performance: A) regions passing cluster threshold correlated with response time in the N-back task. B) regions passing cluster threshold correlated with accuracy in the N-back task. Cluster threshold $z > 2.3$, $p < 0.05$. Red voxels indicate 1-back > 2-back contrast; blue voxels indicate 2-back > 1-back contrast.

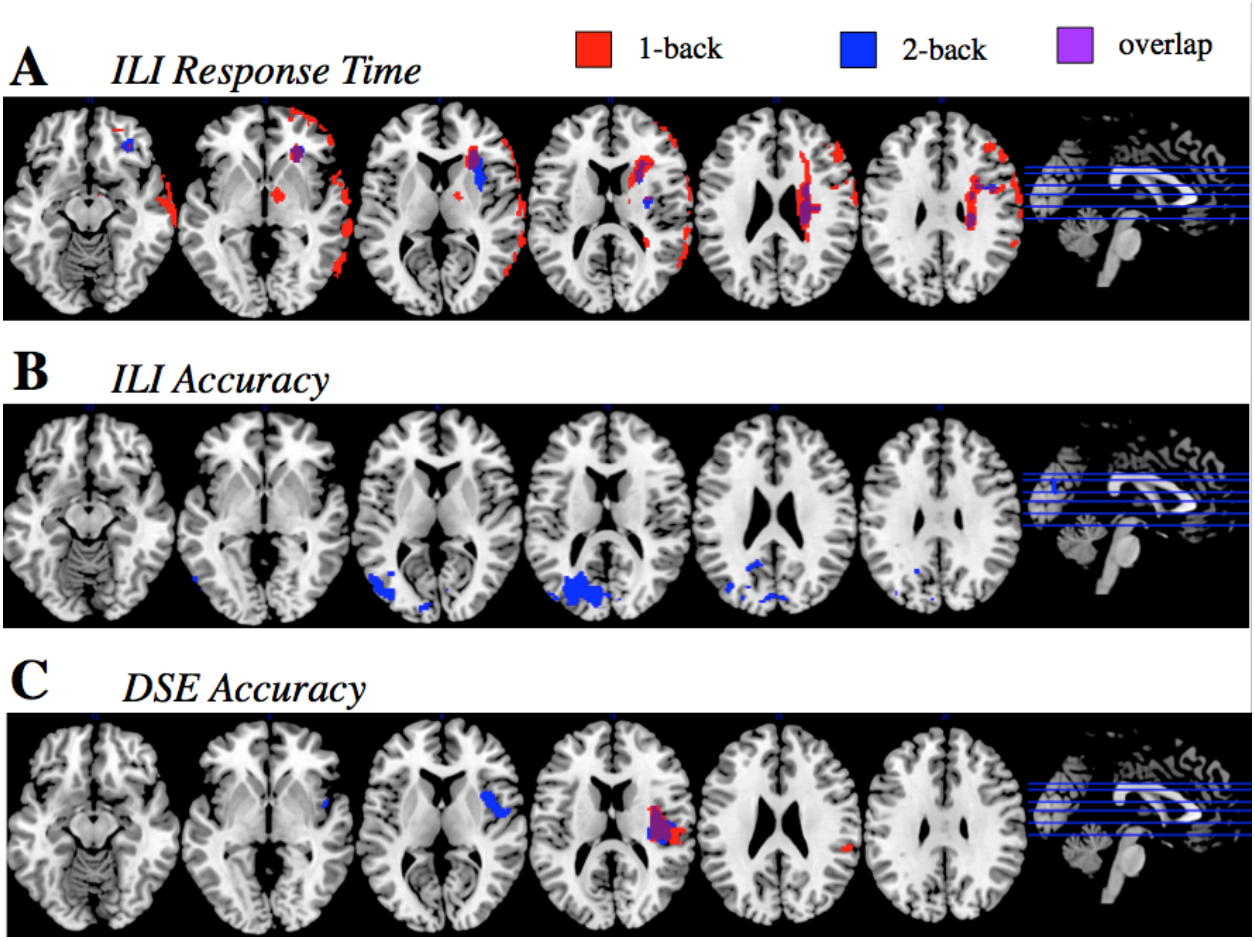


Figure 12: Group differences in regions associated with N-back performance: A) regions associated with response time in the ILI group only. No regions were associated with response time in the DSE group. B) regions assoiated with accuracy in the ILI group. C) regions associated with accuracy in the DSE group. Red voxels indicate associations with the 1-back task. Blue voxels indicate associations with the 2-back task. Purple voxels are regions where activity is significant in both 1-back and 2-back tasks. Cluster threshold $z > 2.3$, $p < 0.05$.

Table 6: Peak voxels associated with N-back performance: Activation is reported in z-scores, coordinates are in MNI space

	Peak Z-score	X (mm)	Y (mm)	Z (mm)
1-back > 2-back				
Response Time *(ILI Group only)*				
r inferior frontal gyrus	5.23	28	28	4
r frontal pole	3.45	54	42	0
r superior temporal gyrus	3.42	70	-14	4
r middle temporal gyrus	3.41	68	-32	-2
Accuracy *(DSE Group only)*				
r insula	3.56	36	-6	14
r parietal operculum cortex	3.46	52	-16	12
r parietal operculum cortex	3.33	48	-26	16
r parietal operculum cortex	3.1	58	-34	24
2-back > 1-back				
Response Time *(ILI Group only)*				
r inferior frontal gyrus	5.3	28	28	4
r insula	3.54	32	10	8
r frontal pole	3.35	32	38	-10
Accuracy				
ILI Group				
l lateral occipital	3.79	-26	-76	18
l lateral occipital	3.53	-48	-78	10
DSE Group				
r insula	3.53	34	-20	20
r parietal operculum cortex	3.21	44	-26	16
r insula	3.2	36	6	10

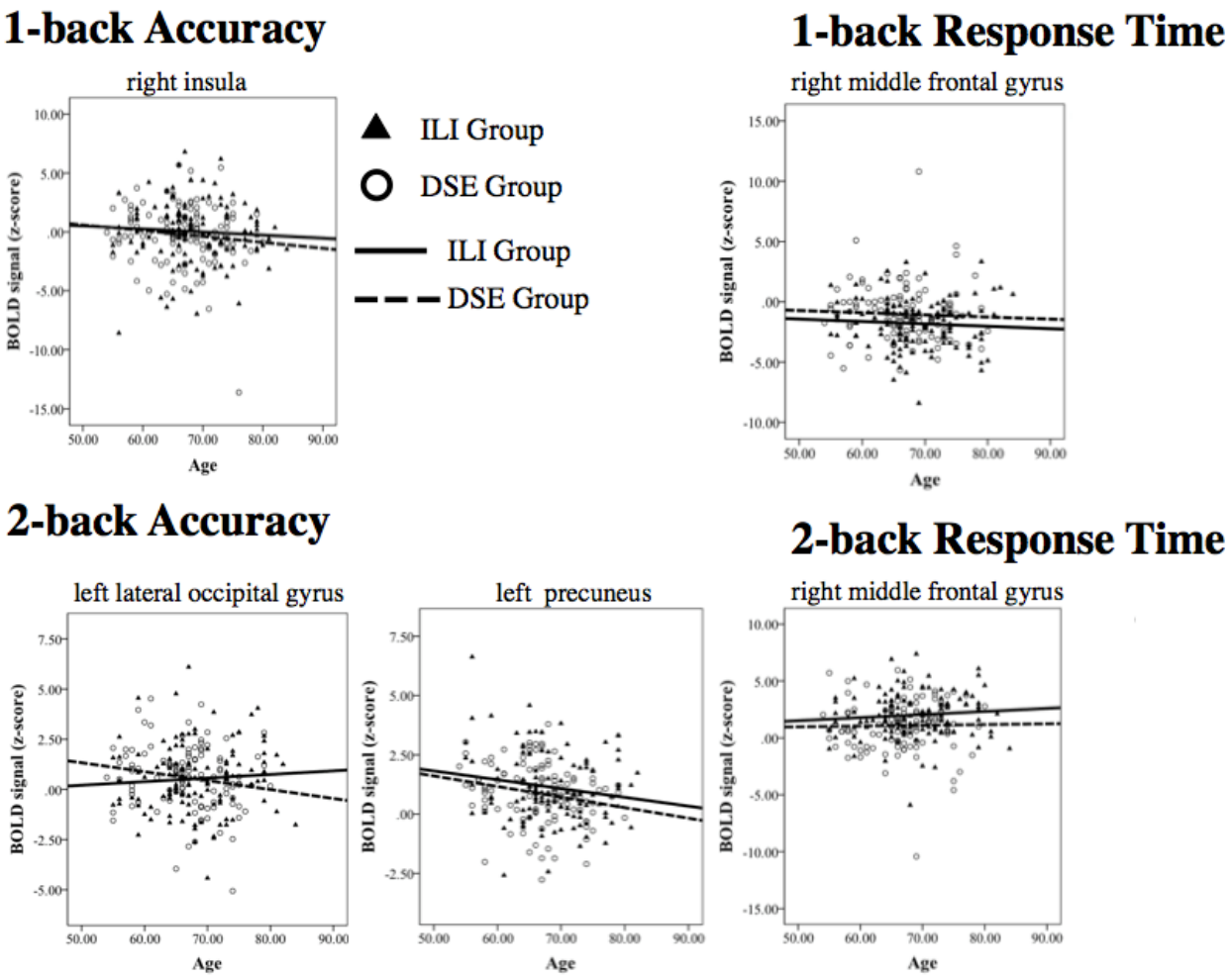


Figure 13: Correlation between age and BOLD signal by group: Scatter plots were fitted with line of best fit. Solid triangle and solid line indicates ILI group. Open circle and dotted line indicates DSE group.

Table 7: Age moderates relationship between year 10 HbA1c and N-back accuracy

1-back Accuracy					
Model		β	t	p	R^2
1	Gender	-0.127	-1.655	0.100	0.005
	Site	0.020	0.263	0.793	
2	Age	-0.265	-3.461	0.001**	0.062
	y10 HbA1c	-0.075	-0.963	0.337	
3	Age x HbA1c	1.470	2.250	0.026*	0.084
2-back Accuracy					
Model		β	t	p	R^2
1	Gender	-0.097	-1.256	0.211	0.008
	Site	0.105	1.366	0.174	
2	Age	-0.327	-4.360	0.000**	0.101
	y10 HbA1c	-0.045	--0.589	0.557	
3	Age x HbA1c	2.274	3.639	0.000**	0.163

* $p < .05$

** $p < .01$

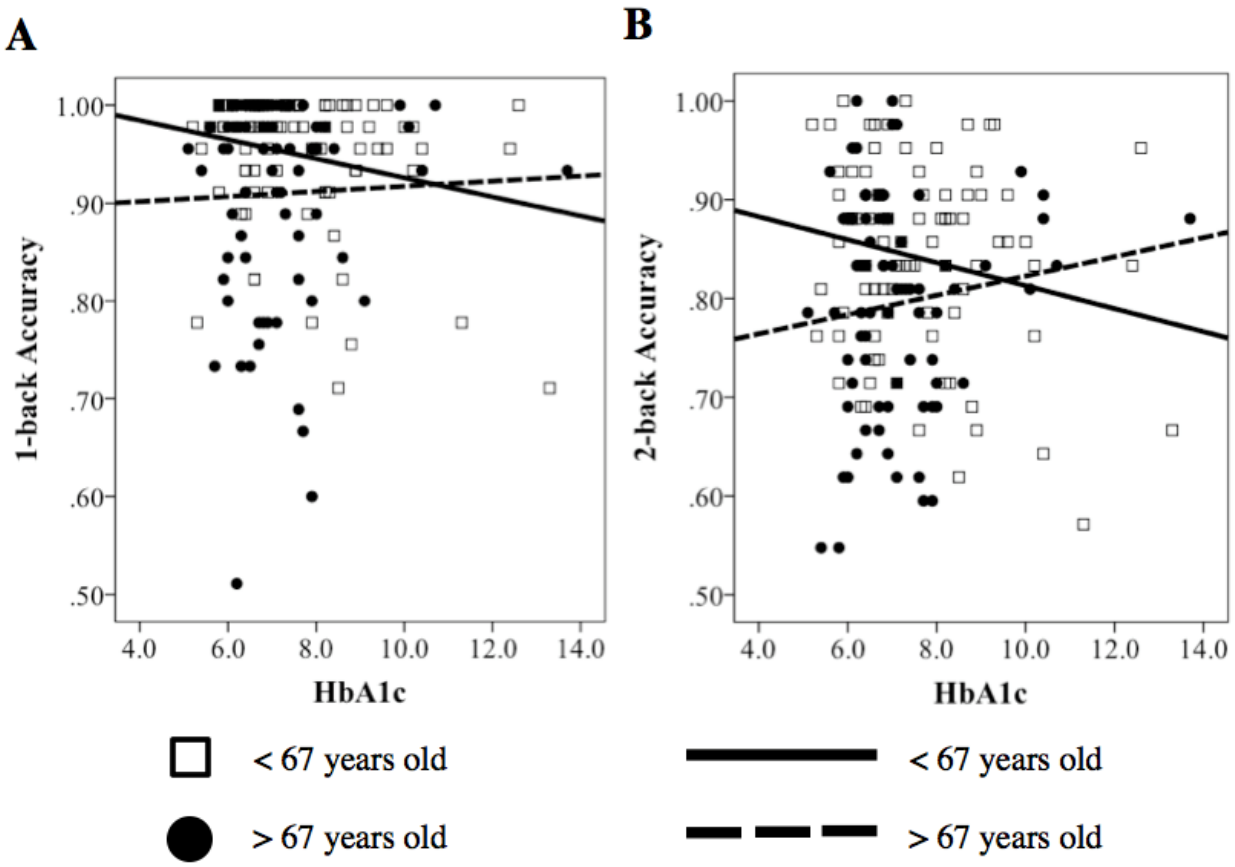
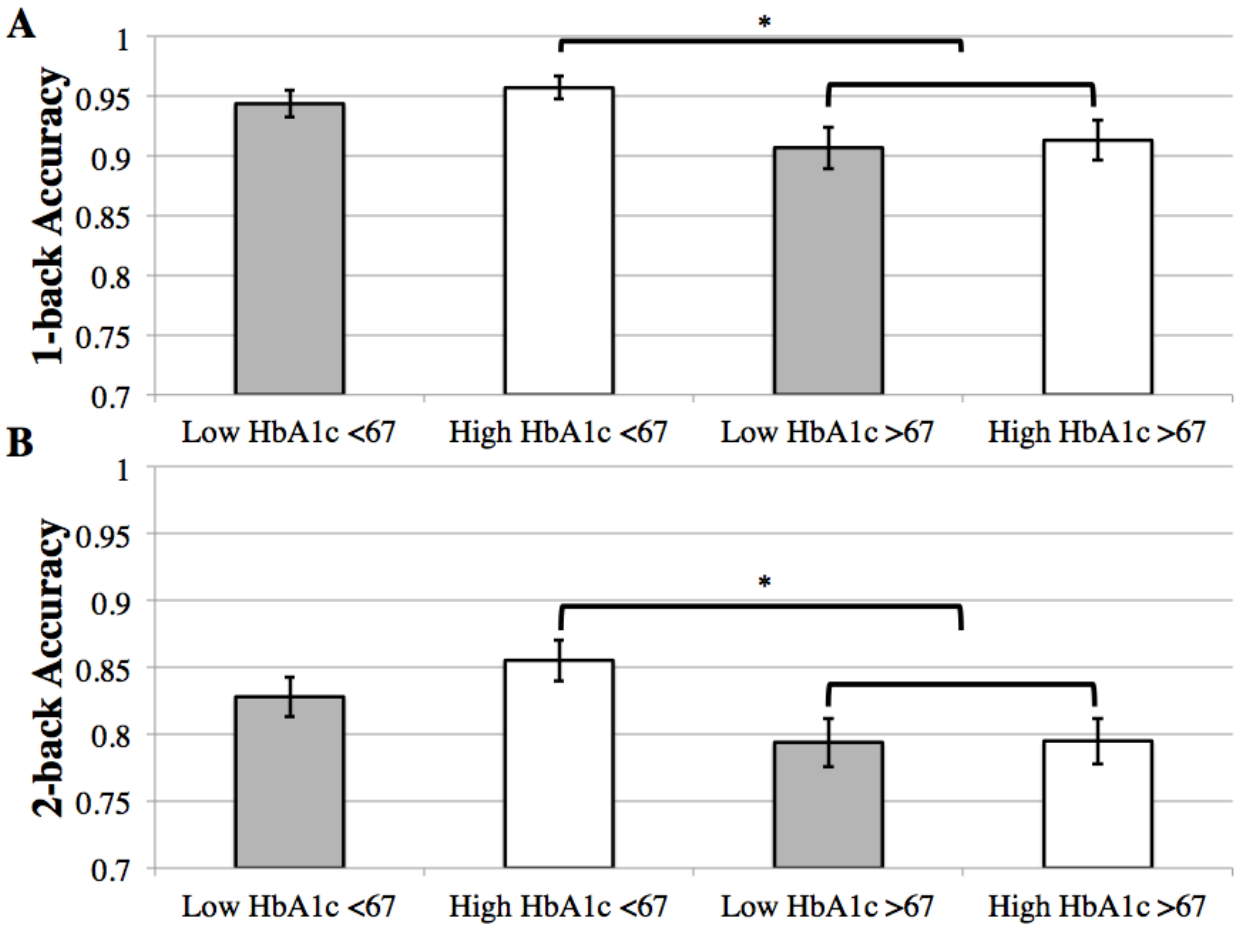


Figure 14: Relationship between HbA1c and N-back performance by age



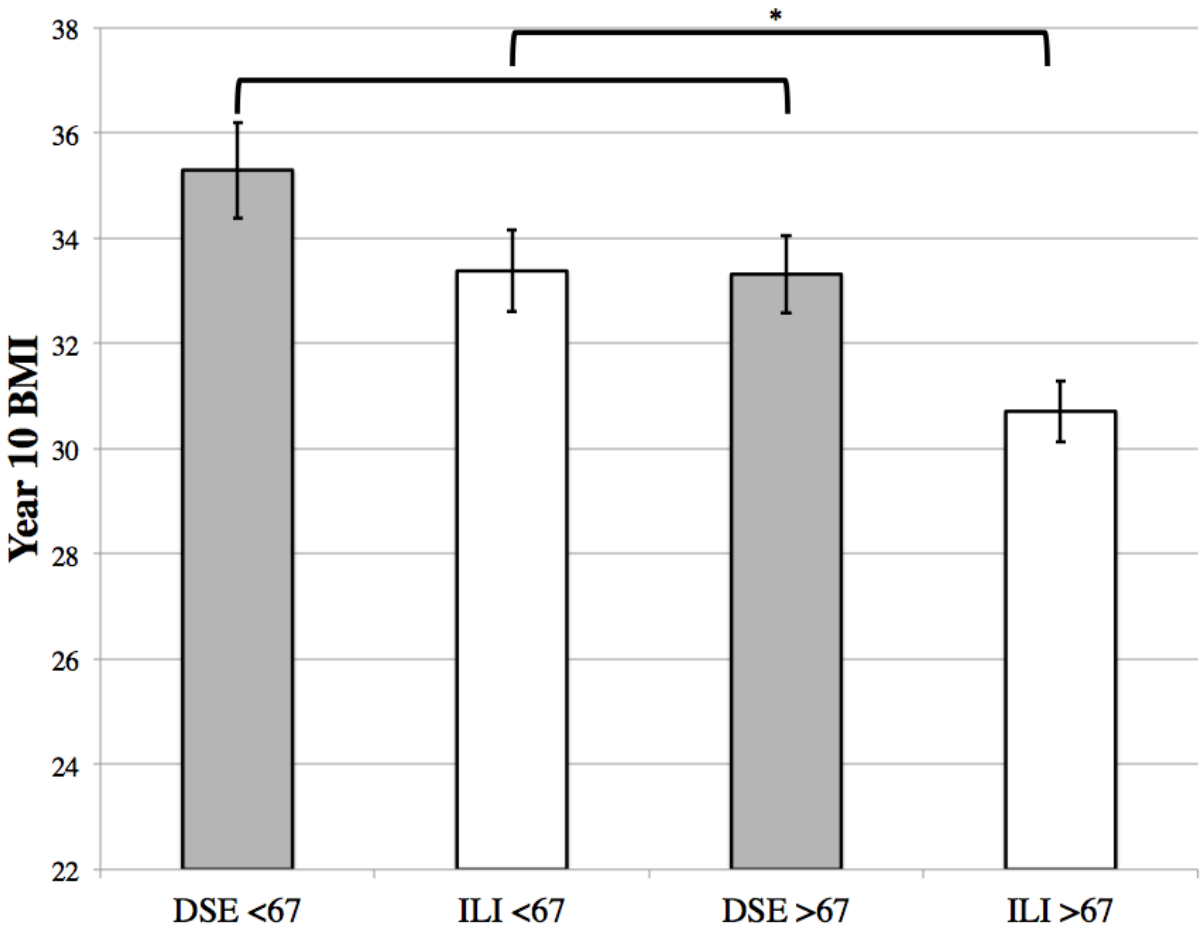
* $p < .05$

Figure 15: Median split of age and HbA1c

Table 8: Age does not moderate relationship between group and year 10 HbA1c

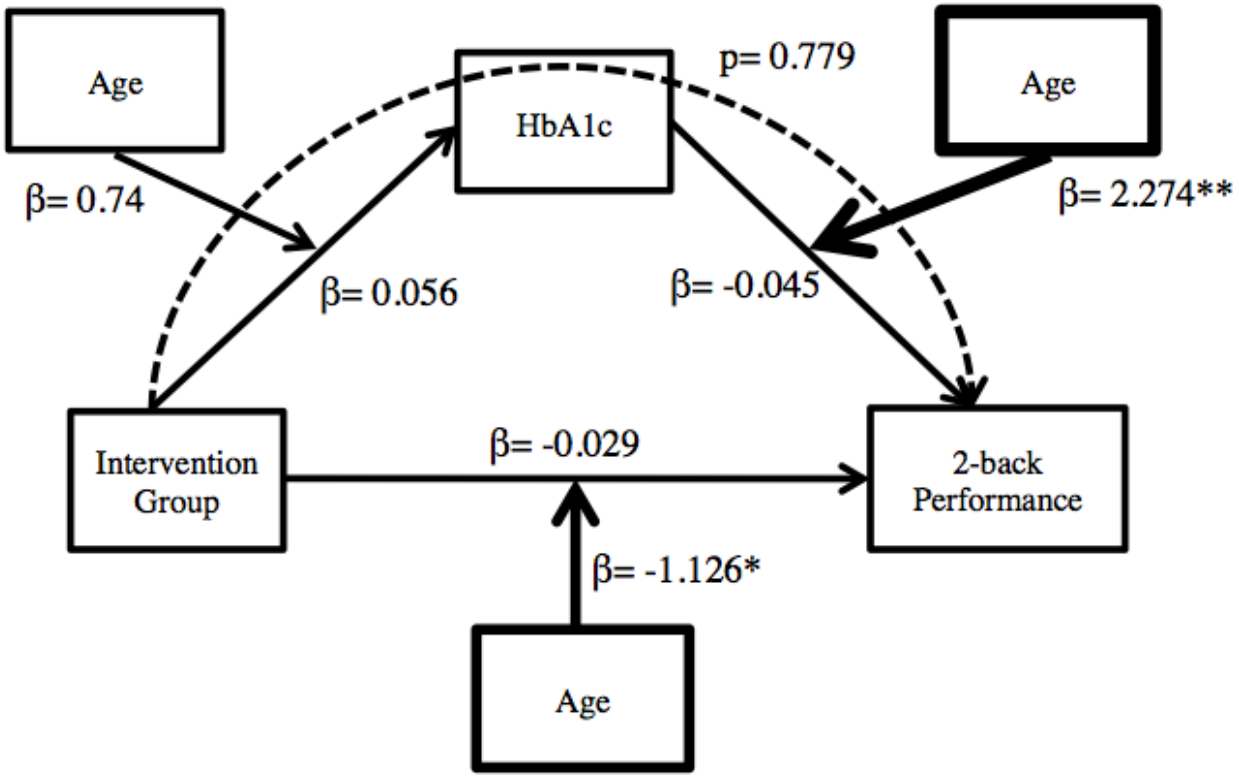
Year 10 HbA1c levels					
Model		β	t	p	R^2
1	Gender	0.108	1.389	0.167	0.035
	Site	-0.154	-1.972	0.050	
	Diabetes Duration	0.006	0.073	0.942	
2	Age	-0.195	-2.516	0.013*	0.078
	Randomization	0.056	0.772	0.472	
3	Age x Randomization	0.740	1.054	0.293	0.085

* $p < .05$



* $p < .05$

Figure 16: BMI differences in age x group



* $p < .05$

** $p < .01$

Figure 17: Summary model of moderated mediation results: Solid lines indicate direct effect. Dotted line indicates indirect effect of mediation. Bold outlines indicate degree of significance. Standard beta coefficients are reported.

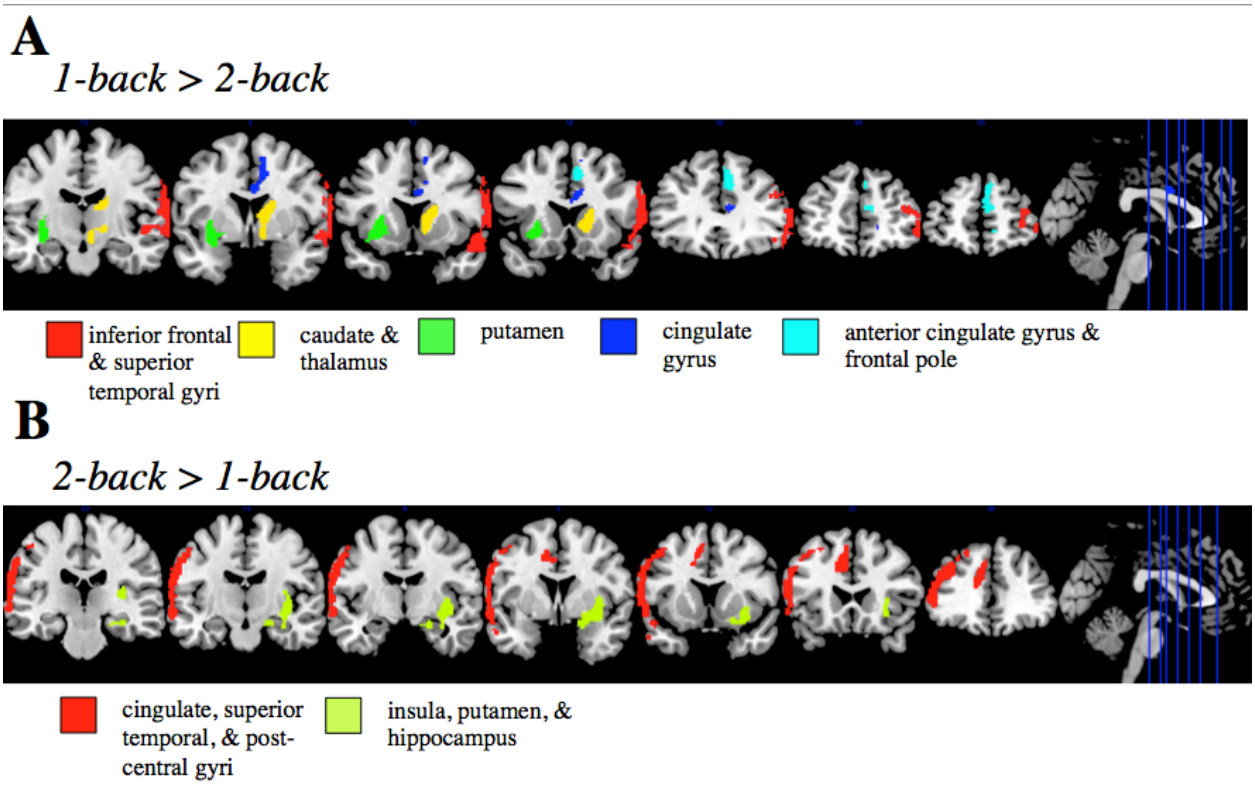


Figure 18: Regions associated with greater HbA1c levels: A) Clusters associated with BOLD signal during the 1-back > 2-back contrast. B) Clusters associated with BOLD signal during the 2-back > 1-back contrast. Cluster threshold $z > 2.3$, $p < 0.05$.

Table 9: Peak voxels associated with HbA1c and activation in the 1-back task

Cluster	1-back > 2-back	Peak Z-score	X (mm)	Y (mm)	Z (mm)
1	r supplementary motor cortex	4.05	12	-4	50
	r cingulate cortex	3.72	4	4	30
	r cingulate cortex	3.48	4	14	26
	r supplementary motor cortex	3.45	10	0	56
	r anterior cingulate cortex	3.38	8	26	18
	r anterior cingulate cortex	3.27	12	0	42
2	r cingulate cortex	3.84	10	20	44
	r frontal pole	3.72	6	56	30
	r cingulate cortex	3.69	8	32	34
	r cingulate cortex	3.37	6	52	16
	r frontal pole	3.2	4	58	14
	r superior frontal gyrus	3.17	6	34	44
3	l putamen	3.53	-32	-6	-4
	l putamen	3.45	-24	16	-2
	l putamen	3.28	-32	-14	-10
4	r caudate	4.24	10	6	10
	r caudate	4.18	12	0	14
	r thalamus	4.05	18	-20	18
	r thalamus	3.37	14	-26	0
	r caudate	3.35	18	24	4
5	r superior temporal gyrus	4.63	68	-30	18
	r temporal pole	4.2	58	26	12
	r inferior temporal gyrus	4.17	58	22	12
	r superior temporal gyrus	4.16	66	-20	14
	r frontal pole	4.1	52	44	-2

Table 10: Peak voxels associated with HbA1c and activation in the 2-back task

Cluster	2-back > 1-back	Peak Z-score	X (mm)	Y (mm)	Z (mm)
1	l cingulate cortex	4.39	-12	24	32
	l inferior frontal gyrus	4.26	-56	22	12
	l postcentral gyrus	4.1	-66	-16	12
	l postcentral gyrus	4.08	-64	-18	28
	l postcentral gyrus	3.97	-64	-10	24
	l frontal pole	3.96	-36	38	30
2	r hippocampus	4.75	34	-18	-16
	r parahippocampal gyrus	4.25	22	-32	-12
	r putamen	4.1	34	-14	-6
	r insula	3.66	34	-20	10
	r insula	3.63	36	-18	4

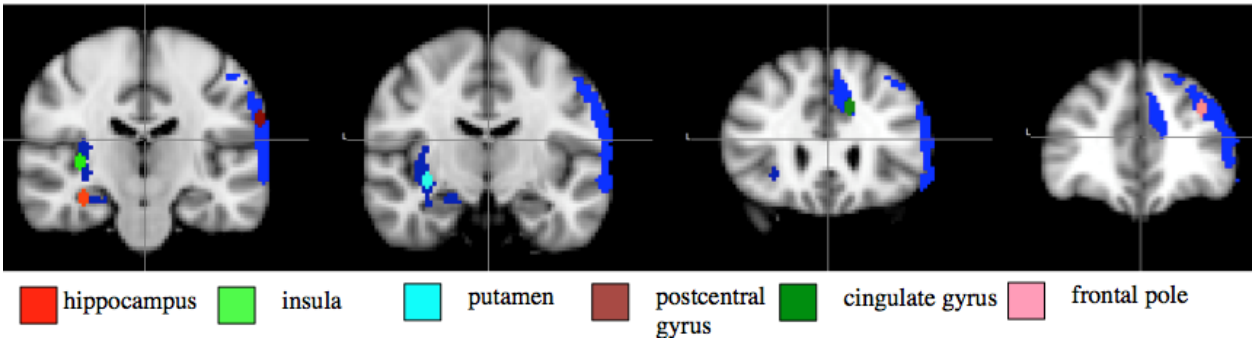


Figure 19: Spherical ROIs: Image is in radiological space, left brain regions are located to the right of the midline.

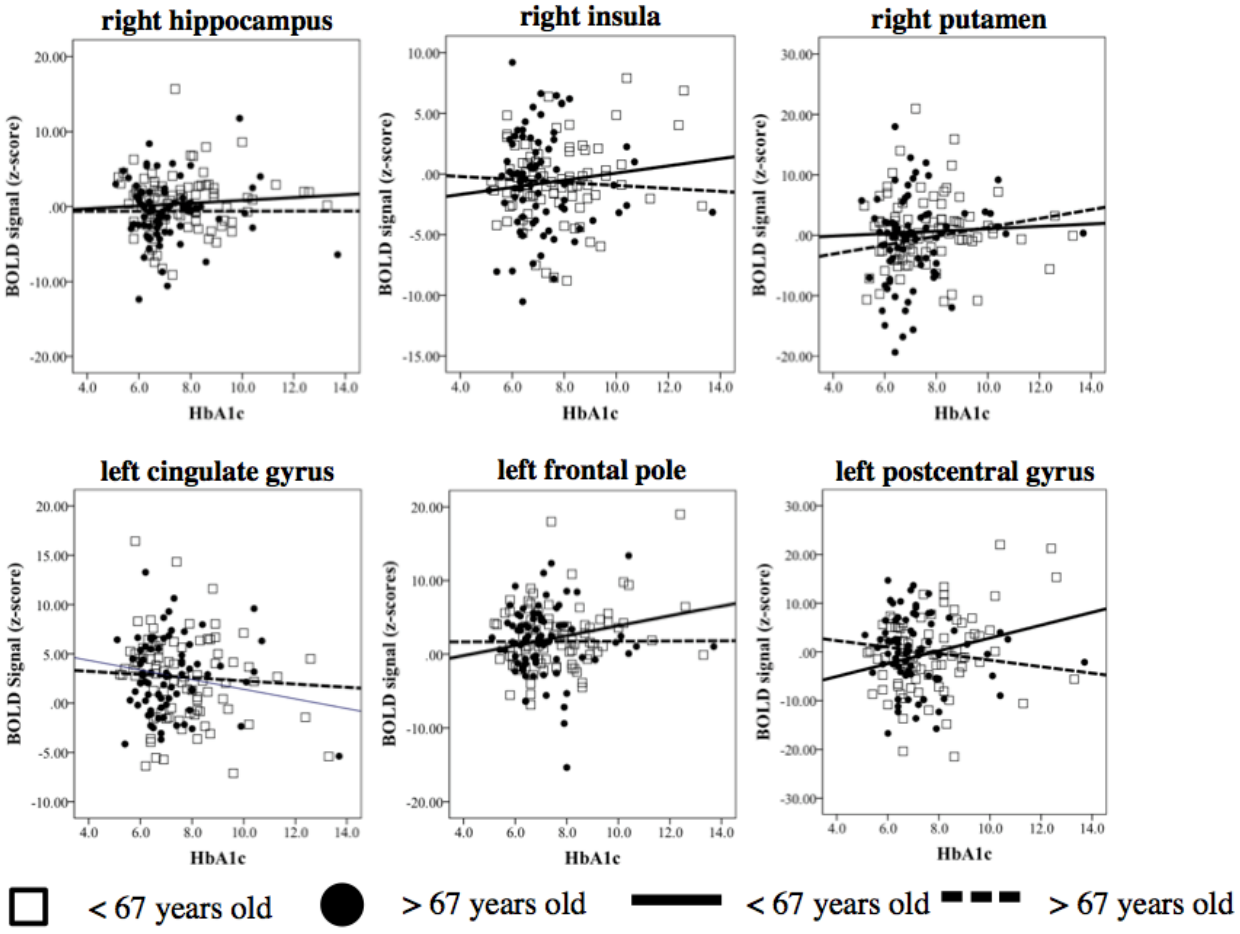


Figure 20: Correlation between HbA1c and BOLD signal by age: Open squares and solid lines indicate adults < 67 years old on the lower end of the median split of age. Solid circles and dotted lines indicate adults > 67 years old on the higher end of the median split of age.

4.0 DISCUSSION

Contrary to my hypothesis, there were no differences in N-back accuracy or response time in either the 1-back or the 2-back between ILI and DSE groups. Similarly, there were no significant differences in brain activity in the 1-back or the 2-back tasks between groups. However, in support of the hypothesis, age did significantly moderate the relationship between group and accuracy on the 2-back task. Interestingly, older adults in the ILI group only demonstrated poorer performance, via lower accuracy on the 2-back, than younger adults in the ILI group. There was no relationship between age and performance within the DSE group. Therefore, something protective was maintaining cognitive performance in the DSE group as the individuals aged that was absent from the ILI group. This direction of relationships is contrary to the hypothesis that the ILI group would perform better, as a result of lasting effects of the intervention. Brain activity did not show a similar relationship, as age did not significantly moderate the relationship between group and activity during the N-back task. As a result, the behavioral outcomes stand alone and either precede any brain changes or are attributed to factors other than activity measured via BOLD signal. In sum, the only hypothesis supported was that age significantly moderated the relationship between group and behavioral cognitive performance, however in an opposite direction of what was anticipated.

Similarly in Study 2, there was no significant difference between ILI and DSE groups on HbA1c levels in year 10, despite a significant difference in BMI. BMI and HbA1c were not correlated, nor was HbA1c correlated with any other measure tested, with the exception of age. Interestingly, HbA1c was negatively correlated with age, meaning that overall older individuals demonstrated lower HbA1c levels. This is contrary to the literature, stating that HbA1c usually increases with age [27], and what was hypothesized. HbA1c did not predict any behavioral measures of the N-back task, such that HbA1c was not associated

with working memory performance. However, there were many regions in the brain whose activity during wither the 1-back or 2-back tasks was significantly correlated with HbA1c levels across the entire sample. In the 1-back task, regions in the inferior frontal gyrus, caudate, thalamus, putamen, cingulate, and frontal pole were identified as correlated with HbA1c levels, such that these areas showed greater activation with higher HbA1c levels. The behavioral data demonstrated that higher HbA1c levels were beneficial to performance, so although higher activation isn't what was predicted in relation to higher HbA1c levels, it is in line with behavioral results from this sample. This same relationship was demonstrated in with the 2-back task, as higher HbA1c levels were correlated with greater activity in regions of the cingulate, insula, putamen, and hippocampus during the 2-back task.

When examining the effect of age on HbA1c between groups, no significant relationship emerged, such that age did not moderate any effect of group on HbA1c levels. However, in support of my hypothesis, age significantly moderated the relationship between HbA1c levels and accuracy on both the 1-back and 2-back tasks. Interestingly, the direction of this relationship was such that older individuals and individuals with lower HbA1c (better glucose control) had significantly lower accuracy scores than younger individuals with higher HbA1c (worse glucose control) scores. This is again contrary to what was expected, as the literature suggests that higher HbA1c should result in lower accuracy [23, 43, 94]. However, brain activity during the N-back task did not reveal any region where age moderated the relationship with HbA1c.

To confirm this further, brain activity was extracted from peak regions associated with HbA1c levels during the 2-back task. Activity was then entered into a moderation model with age and HbA1c and confirmed that age did not significantly moderate activity in any of these regions (right hippocampus, right putamen, right insula, left cingulate, left postcentral gyrus, left frontal pole). However, when graphing activity against HbA1c levels and separating by age, it appears that adults <67 years of age tended to have higher activation in conjunction with higher HbA1c levels. This trend was not statistically significant, but is still interesting in that it is the opposite of what was predicted.

Finally, the main hypothesis that HbA1c would mediate the relationship between intervention group and N-back performance as a function of age was not supported. Average

brain activity from significant regions associated with N-back performance was extracted and entered into the model, yet HbA1c did not mediate any relationship between group and brain activity in any of these regions.

The results of the current study are surprising and in many ways contrary to the proposed hypotheses and directions of predicted relationships; however, age emerged as a critical variable of interest in the relationship between both intervention group and HbA1c with cognitive performance. In this sample, adults over the age of 67 with lower BMIs and lower HbA1c levels who took part in the Intensive Lifestyle Intervention display worse working memory performance than individuals under the age of 67 with high HbA1c levels. These results highlight that not only does glucose control differentially affect working memory in older adults, but that higher BMI seen in the DSE group and adults less than 67 years of age may be protective to working memory function.

4.1 IMPACT OF INTENSIVE LIFESTYLE INTERVENTION ON WORKING MEMORY IN YEAR 10

At year 10, there were no significant differences between the ILI and DSE groups on any behavioral measure of working memory performance, glucose control, or weight. The lack of group differences in cognition mirrors findings from Espeland and colleagues [36] at year 8 of the Look AHEAD study. The authors administered a series of cognitive tasks to 978 participants during their year 8 follow-up and found no significant differences between ILI and DSE groups on performance in any cognitive domain, including working memory. This suggests that any benefit that the intervention may have had on cognition did not persist after the participants were left to continue to lose weight through better diet and more physical activity on their own. However, the authors did not look for any interactions with age. Age had a significant impact on group differences in working memory performance in the current sample; therefore, it is possible that this relationship exists in the authors' sample as well.

4.1.1 Age as a moderator

In the current study, age was a significant moderator of the relationship between group and N-back accuracy, such that adults > 65 years old in the ILI group demonstrated poorer accuracy than adults < 67 year old. What is important here is that this relationship was only present in the ILI group, meaning that there was no difference in performance across age groups in the DSE group. This can be interpreted in two different ways; first, older adults in the ILI group are demonstrating cognitive dysfunction, or second, that adults in the DSE group are maintaining cognitive function despite typical age-related declines. This second interpretation is most interesting, as the age range of DSE participants goes to 80 years of age.

Working memory performance declines with age [49, 81] and this trajectory is reported to increase in T2DM [100]. Additionally, the average response times in the current sample are below averages reported in a similar aged (mean age = 63.7 years) adults with T2DM [19]. Therefore, it is less likely that just the older participants in the ILI group are showing decline, but more likely that the older participants in the DSE group are somehow maintaining cognitive function.

There are several reasons why the control group may be protected from further decline with age. The first possibility is that the participants in the DSE group had higher average BMI than those in the ILI group. While seemingly counter-intuitive, adipose tissue can be protective of cognitive function in late life [26]. For example, results from the Framingham Heart Study demonstrated that obesity was not associated with age-related cognitive decline in women [34]. When examining average BMI for each group and within the median split of age, all groups are obese, however many participants > 67 years old in the ILI group have BMIs below 30, despite a mean BMI of slightly over 30 for the group.

Specifically targeting older adults, Buchman and colleagues [17] followed individuals for 5.5 years, assessing global cognition and diagnosis of Alzheimer's disease. The authors concluded that lower BMI was associated with both cognitive decline and increased risk of Alzheimer's disease. Interestingly, the high risk group had an average BMI of 25.8, while the low risk group had an average BMI of 27.8. These values are lower than the average BMIs

in the current study, but it is possible that working memory is more sensitive to BMI than overall cognition and that there is a shift in effect of BMI in T2DM.

4.1.2 Interpreting fMRI results

While there was no main effect of group on brain activity during the N-back task, the regions associated with the N-back varied between the groups. The ILI group showed significant activation in left lateral occipital regions associated with 2-back accuracy. However, the DSE group shows more activation in the right insula, parietal operculum cortex, and superior temporal cortex associated with 2-back accuracy. Therefore, better accuracy in the ILI group is not associated with any of the same regions than those associated with the DSE group. These differences are particularly interesting because regions in the DSE group, such as the superior temporal cortex, are anatomically close to regions in the prefrontal and inferior frontal cortices that are traditionally associated with N-back accuracy [15]. However, lateral occipital regions have not, to date, been associated with accuracy on the N-back task.

What is particularly compelling about these results is that it seems that the DSE group, despite having higher BMI, show brain activity expected in a non-diabetic adult [77]. Still, comparing results from the current sample to other reported N-back performance measures in T2DM [19], both the DSE and ILI groups had slower average response times on the 1-back and 2-back. That is, the current sample was not a high-functioning group, which would explain the "normal" brain activation patterns. Therefore, it seems that the DSE group holds some characteristic that is protective and is maintaining brain activity.

4.2 ROLE OF GLUCOSE CONTROL IN WORKING MEMORY

In this sample, there was no evidence that suggested that glucose control shared a direct relationship with working memory performance, rather this relationship is moderated by age. It was hypothesized that individuals with better glucose control, via lower HbA1c levels, would have better working memory performance, via shorter reaction times and higher

rates of accuracy. Because no group differences were present in HbA1c, it was unlikely that there would be a main effect of group on working memory performance, and in fact, this was the case. However, HbA1c varies between individuals in the current sample, supplying a range from 5.1%−13.7%. Therefore, it was hypothesized that among all participants, HbA1c should predict working memory performance, such that higher HbA1c would be associated with poorer performance. This hypothesis was not supported in the behavioral data. After breaking up the sample into tertiles based on HbA1c levels, further examination suggested that individuals with the highest HbA1c levels were actually performing better than individuals with lowest HbA1c levels.

These findings are contrary to much of the literature, which has long reported that glucose control is negatively associated with cognitive function, such that high HbA1c levels are associated with poor cognition [86, 90, 112] and increased incidence of cognitive impairment [74, 120]. While unanticipated, the current results illustrate that there is a complexity to the relationship between glucose control and cognition, especially in older T2DM adults. One such complexity that is not accounted for in previous studies is the individual differences associated with age. Most reports of HbA1c and cognitive function use age-adjusted cognitive scores or age-adjusted HbA1c levels. The reasoning behind this is that age is associated with both HbA1c and cognition. For the first time, the current study examines the role that age plays in the relationship between glucose control and cognition, revealing new information on the importance of age in T2DM and cognition.

4.2.1 Age as a moderator

Age plays a significant role in the current sample when examining the relationship between HbA1c on working memory. Age moderated the effect of HbA1c on N-back accuracy, such that older individuals with lower HbA1c levels demonstrated lower accuracy. There was also a main effect of age, such that older individuals demonstrated poorer accuracy than younger individuals. This relationship is contrary to what is expressed in the literature, where better glucose control is associated with better cognitive performance in older T2DM adults [41, 55, 60, 73, 90, 102].

One possible variable to consider when interpreting the effect of age on the relationship between HbA1c and cognition in older adults is diabetes duration. In the literature, diabetes duration is associated with cognitive performance, such that longer durations are associated with poorer performance. West and colleagues [117] are the first to examine HbA1c as modulating this relationship, reporting that diabetes duration best predicted working memory function in individuals with high HbA1c levels. That is, individuals with both long diabetes duration and high HbA1c levels displayed the poorest performance. Yet, in the current findings, duration of diabetes did not contribute to the relationship between age, HbA1c, and working memory performance. In fact, there were no main effects of diabetes duration on any measures assessed here including fMRI activity or n-back performance. One explanation for this discrepancy is that, while West and colleagues [117] had a sample with an average age of 72 years, they also had a smaller range of HbA1c levels (4.0%−10.1%) and had a predominantly male population (59.3%). The current sample is of a similar age, but with a wide range of HbA1c levels and a mainly female population. In addition, West and colleagues [117] included age as a covariate in their model, but do not report their hierarchical model, where regression coefficients and R2 values would be reported for each variable entered into the model. This eliminates any possibility of identifying a main effect of age, and it is unclear how much variability in working memory performance or HbA1c levels was accounted for by age. Therefore, it is difficult to determine if age and HbA1c are related in their sample, such that those with higher HbA1c in older age perform better than those with lower HbA1c in older age.

Another possible interpretation is that the current study boasts a larger age range and, on average, older participants than other studies examining the effects of HbA1c on cognitive function. However, results from The Rotterdam Study report better cognition in association with lower serum glucose levels in a population averaged ∼69 years with a sample of over 5,500 participants [102]. Similarly, Reaven and colleagues [90] report that in a sample of adults mean age 69.8 years old, lower HbA1c is associated with better cognitive performance in T2DM. Therefore, it is difficult to determine that the current sample is unique in its age range and thus explain why the current results are opposite from previously published studies.

In relation to the age range of the current study, it should also be noted that the average lifespan of an individual with T2DM is 8 years less than the national average of 78.8 years [75]. This is, if the life expectancy for an individual with T2DM is 70.8 years, and the current sample has a range that surpasses this number, then there are likely individual differences that you contributing the health and longevity that may interfere with predicted effects of HbA1c on cognitive performance.

4.2.2 Interpreting fMRI results

HbA1c levels were associated with brain activity during the N-back task in several regions. Regions in the right hippocampus, right putamen, right insula, left cingulate, left postcentral gyrus, and left frontal pole were all associated with activation during the 2-back task after removing activation associated with the 1-back task. While these regions, particularly the hippocampus, cingulate, and frontal pole are associated with the N-back task in the literature [15, 77], it was hypothesized that there would be less activation associated with higher HbA1c. This hypothesis was based on evidence that individuals with T2DM demonstrated lower activation during the N-back task than non-diabetics and that this relationship might be a result of poorer circulation and higher HbA1c levels in T2DM [19]. However, since the formation of the hypothesis of the current study, Zhang and colleagues [122] reported that older adults (mean age 54.15 years) demonstrated higher activation in the bilateral postcentral gyrus, supramarginal gyrus, dorsal anterior cingulate gyrus, right superior temporal gyrus and right insula, among others, was significantly higher in T2DM compared to non-diabetics. The diabetic population had an average HbA1c of 7.87% compared to the control's 5.55%. While the authors did not test the effect of HbA1c directly, this study provides some evidence that higher HbA1c might result in increased activation during a working memory task. Of note, is that while the current study only found lateralized regions, many of the reported regions overlap with the regions identified by Zhang and colleagues [122].

One possible explanation for the unexpected greater activity associated with HbA1c is that hyperactivation is sometimes an indication of injury or lack of efficiency, particiularly in older adults [91]. However, HbA1c was associated with better accuracy in the N-back task

and therefore, it is much more likely that the greater activation observed is contributing to better cognitive performance, rather than an indication of potential cognitive decline. In fact, Zhang and colleagues [122] report that connectivity in the fronto-parietal regions in the T2DM adults was associated with better accuracy, and conclude that there are likely compensatory mechanism at play in T2DM that maintain working memory performance. The current study suggests that this relationship is present in older T2DM, such that without higher HbA1c and BMI, compensatory mechanisms are removed and results in poorer working memory function. Unfortunately, there is no clear indication of what such mechanisms may be, nor is there any literature that points to a protective effect of HbA1c, as it has never been observed in the literature.

To speculate, it is possible that individuals with T2DM have adapted to high levels of HbA1c over time, such that by the time they reach old age, they are somewhat resistant to the negative effects of HbA1c. This speculation is based on the brain's ability to adapt to serious physical injury [4] and the fact that these participants are still living without dementia, meaning that if there were large and irreversible effects of chronic high levels HbA1c on the brain it is unlikely that anyone with T2DM would remain cognitively normal in old age.

4.3 LIMITATIONS

The largest limitation to the current study is lack of group differences in glucose control at year 10. It is impossible to maintain an intervention indefinitely, however the current study would have a much larger impact if the intervention groups remained distinct from each other in measures of glucose control and diabetes management. This is because group differences would provide information on the effect of physical activity, weight-loss, and caloric restriction on HbA1c levels in one sample, as well as provide insight into how these changes result in differences in working memory function and brain activity. However, following the study participants so long after the active intervention has ceased, and continuing to collect new data, provides valuable information on the longevity of such an intervention.

Another major limitation is that the two groups ceased to be significantly different from each other in weight and BMI by year 4 of the intervention [113]. In fact, results indicate that, while the ILI group lost a significant amount of weight at year 1, they slowly began to gain weight back over the next 3 years. At the same time, the DSE group was slowly losing weight so that by the 3rd and 4th years, their average weight and BMIs were similar to those of the ILI group. Based on this trajectory, one can then assume that any effects of the initial intervention would have to be residual after the first few years. Indeed, it is difficult to identify exactly which aspect of the intervention is driving the relationship between group and performance as a function of age without measures of physical activity, fitness, or diet profile in year 10. Additional information on mortality between groups may also assist in identifying the lasting effects of the intervention and how it is moderated by age.

A related limitation to the current study is the presence of an MRI scan and working memory performance at year 10 only. This means that all data can only be interpreted cross-sectionally and change in working memory performance cannot be assessed over time. If participants completed an MRI scan at baseline, year 1, and year 10, for example, it would be possible to determine the impact of the intervention on working memory performance and brain function both at the height of the group differences and long after the lifestyle modifications waned. Yet, the current study sets precedence for future studies to use neuroimaging when examining lifestyle interventions. It also draws attention to the multitude of factors affected by an alteration in lifestyle, such as cognition and brain function, aside from the traditional variables of interest of cardiovascular health and body mass.

One limitation inherent to MRI is that only one cognitive domain was examined, due to cost and value of time during an MRI scan. In order to separate brain activity associated with a cognitive task, the task needs to be modified from its traditional presentation, (i.e., administered via paper and pencil) and transformed into a computer-based task of one of three possible designs. MRI functions by collecting snapshots of the brain every 2 seconds or so, meaning that stimuli must be presented in a way that one snapshot every 2 seconds would capture the entirety of the cognitive process in question. This typically requires stimuli to be presented many more times than is necessary for behavioral testing, and therefore, cognitive tasks can take a long amount of time to complete in the MRI environment. However, MRI

time is expensive, with costs billed in 30-minute increments and often many additional sequences are required in order to maximize the use of the MRI data. Therefore, it is common to only test one or two cognitive domains in one MRI session. In the current study, working memory via the N-back task was chosen. Working memory is a domain that has been associated with T2DM in pervious literature [96] and the N-back task is a well cited, reliable, and sensitive measure of working memory [53]. Thus, this was the cognitive domain of interest. In an ideal world, multiple domains would be examined, as it is known that working memory is not exclusive in its relationship to T2DM [78].

One final limitation to MRI studies is the necessary population. Specifically, participants must meet a series of criteria in order to be eligible for the MRI. In the current sample, 29.5% of available participants were not eligible for participation due to screening criteria. The criteria states that participants cannot have any metal implants, including pacemakers, stents, or eye lenses, for example. This is of greater importance in the current sample because the sample is Type 2 diabetic; this is a population that is at greater risk for heart disease that requires stents and pacemakers and retinal or ocular degeneration that requires lens implants. Therefore, the MRI screening criteria eliminate a very representative population of T2DM patients, leaving only the healthiest individuals eligible to participate. To summarize the previous argument, the individuals in the current analyses are not generalizable to the T2DM community due to their health status, or lack of health problems, that allowed them to participate. This is a limitation for all future MRI studies with T2DM populations and should be considered when interpreting results.

Unfortunately, significant differences were found between data collection sites both in working memory performance and brain activity during the task. This is a limitation because it requires site to be added as a covariate in the model, reducing degrees of freedom, but also because it indicates that the task and MRI were not administered in the same way across sites. This limitation hinders interpretation, to a degree, in that it is possible that task instructions were unclear at some sites and participants were not actually performing the task correctly. It could also be a function of population, as Philadelphia, Pittsburgh, and Providence are entirely different cities. It is possible that the communities differ enough to show differences in cognitive performance. Interestingly, 30 of the 35 participants that

needed to be removed from the analyses due to accuracy <50% were from the same site. It is possible that the task was incorrectly administered, but it is also possible that the site in question recruited and had access to a population with higher rates of Mild Cognitive impairment (MCI) or dementia. These were no screening factors for eligibility and are possible contributors to site differences. Unfortunately, there is no clear way to identify exactly what caused the site differences.

The exclusive use of HbA1c as a measure of glucose control is also a limitation in the current study. The Look AHEAD study has blood samples collected from many years and available for multiple tests; however, at the time of analysis, only HbA1c was available in the majority of participants. Other variables of glucose control, such as insulin levels, glucose levels, and homeostatic model assessment (HOMA), would further illuminate the role of glucose control on cognition and brain function. HOMA, for example, is calculated from fasting glucose and insulin levels in the blood. What makes HOMA interesting is that is a known and reliable measure of insulin resistance [54], an important factor in glucose control. Insulin resistance is also associated with cognitive performance in T2DM and hypothesized as a possible mechanism by which T2DM display poorer cognitive function [23]. Therefore, adding HOMA levels would have greatly enhanced the interpretation of the role of glucose control on cognitive function in this T2DM population.

Another limitation regarding measure availability is the lack of any objective physical activity measurement at year 10. Studies suggest that physical activity advice, in combination with dietary advice, is associated with reduction of HbA1c in T2DM [108]. However, the authors state that structured physical activity is much more effective in HbA1c reduction. The ILI group in the current study was given physical activity goals and dietary guidelines, but did not participate in any recorded structured physical activity. While fitness was assessed at year 4, an objective measure of regular physical activity might assist in understanding the differences between the older adults in the ILI and DSE groups. For example, if the DSE group was more physically active than the ILI group, that may explain their better cognitive performance.

4.4 FUTURE DIRECTIONS

The Look AHEAD study is vast with fruitful data and many years of data on dedicated participants. Because of this, there are countless directions to follow in future analyses. The most transferrable and potentially impactful with the current study will be discussed here.

First, the completion of blood analyses will also increase the sample size, such that all participants will have HbA1c levels at year 10, adding power to future analyses. Additionally, HOMA values will also be available for the sample. Therefore, one important step would be to examine the relationship between HbA1c and HOMA and cognitive function in all 237 participants. Results from this would clarify any relationship seen as a result of glucose control versus insulin resistance. While these two processes are similar, they are functionally distinct and the majority of the literature points to insulin resistance as a key player in the relationship between T2DM and cognition [23, 44, 68]. The next step with the current sample would be to look at physical activity. A subgroup of the participants brought in at year 10 also consented to wear a pedometer to measure daily steps, or physical activity. As stated in the limitations section above, adding physical activity to model would help in interpretation and provide a bigger picture of the characteristics of this population.

There were also many MRI sequences collected during the current study that have yet to be examined in relation to HbA1c and working memory performance. One article has been published exploring the relationship between grey matter volume and white matter hyperintensities between ILI and DSE groups. Results indicate that the ILI group had similar grey matter volume as the DSE group, but significantly less white matter hyerintensities [37]. The authors covaried fro BMI in the reported analyses, but relationships between structural MRI measures and glucose control as well as working memory performance are interesting next steps. For example, if higher HbA1c levels predict greater grey matter volume in the prefrontal cortex of older adults, hippocampus, and insula, then this would support the current study's findings that higher HbA1c levels are beneficial for T2DM in old age. Alternatively, if high HbA1c levels are associated with less grey matter volume, then perhaps the higher brain activity reported in the current study is an example of hyperactivity indicating injury and less efficient processing.

One final future direction that would assist in the interpretation of the current results is to examine cerebral perfusion or blood volume in relation to HbA1c, intervention group, and age. One consistent effect of T2DM is impaired circulation, particularly in the brain [106]. HbA1c is a measurement of glucose control in the blood, therefore the status of circulation in the brain is relevant to any effects of HbA1c. In addition, fMRI relies on the BOLD signal, which is a direct measure of oxygenated blood in the brain. Therefore, less overall perfusion would impact BOLD signal and its reliability.

4.4.1 Future studies

The current study sets a precedence for future studies to identify age as a variable of interest, rather than a variable whose variability needs to be removed from the sample before conducting analyses. That is, age should not be treated as a covariate, but as a potential moderator for many relationships observed in T2DM. For example, age may moderate the relationship between weight-loss and cognitive performance in T2DM, if higher BMI is protective in older adults.

In addition, future studies would benefit from examining a larger age range. It is very typical for studies to examine only children, adolescents, middle-aged populations, or older adults. Combining these ages into a long range will allow the effect of age to be identified without a 50 year longitudinal study. That is, utilizing the same measures within a T2DM sample of various age ranges, such as HbA1c, HOMA, BMI, blood pressure, and working memory, will provide further evidence for the effect of age throughout the lifespan.

Finally, future studies on T2DM populations should continue to utilize the most sensitive measurements of cognitive function. This means abandoning global measures of cognition, and focusing on computer-based tasks with precise response time reporting, stimulus presentation, and devoid of many aspects of researcher bias. Standard neuropsychological tasks are typically paper-and-pencil, quickly administered, and have been fully standardized over the last 20+ years. The problem is that these tests are most likely not the most sensitive measures of each cognitive domain. Most standard neuropsychological batteries were developed in clinical settings with the intent to quickly and objectively diagnose neurological

impairment. As a result, these test sets show ceiling effects in non-clinical populations [104] and middle-aged participants [1]. However convenient, these measures are not designed to be sensitive enough to detect differences in normal cognition. For example, epidemiological studies examining the relationship between working memory and T2DM or insulin resistance almost exclusively use the Digit Span or traditional neuropsychological tests. The effect sizes seen in these tasks vary, especially in middle aged participants and non-clinical settings [24]. Conforming to common sensitive computer-based tasks can not only have the potential to detect currently unidentified effects of T2DM on cognition, but also may alleviate inconsistencies in the literature regarding the possible mechanisms behind the relationship between T2DM and cognitive performance.

4.5 SUMMARY

In the current sample of older T2DM participants of the Look AHEAD study, no direct effects of the Intensive Lifestyle Intervention on working memory function were identified. However, age significantly moderated the effect of group on working memory accuracy, such that adults $>$ 67 years old in the ILI group performed worse than adults $<$ than 67 years old. This relationship is not in the hypothesized direction, however older ILI group demonstrated lower BMIs than the DSE group and therefore BMI may be protective to cognitive function in older T2DM adults.

There were no main effects of group on HbA1c, contrary to the hypothesis that the ILI group would display lower HbA1c levels. In addition, HbA1c did not mediate any relationship between group and working memory performance. However, age significantly moderated the effect of HbA1c on working memory accuracy, such that lower HbA1c levels in adults $>$67 years old in the lower accuracy than adults $<$ 67 years old. Again, these results indicate that there is something protective or adaptive in high HbA1c in old age. MRI data identifies no group differences or effects of age on brain activity during the working memory task, but DSE participants show activation patterns similar to those of non-diabetic adults. This, too, points to a possible protective or adaptive element of higher BMI in brain function in T2DM.

Higher HbA1c levels were also associated with greater activation in brain regions associated with working memory in T2DM.

Together these results paint an interesting story focused around age and its relationship with T2DM in old age. While higher HbA1c and BMI are associated with poorer cognitive function in the literature, it appears that in older adults, specifically over the age of 67 in this sample, higher HbA1c and higher BMI results in better cognitive performance. The current study is the first to examine the relationships between an Intensive Lifestyle Intervention on working memory function as a factor of age. Similarly, it is the second study to report higher HbA1c levels associated with brain regions active during a working memory task, but the first to examine this relationship as a function of age.

The current study sits as an example of the potential impact of age on the relationship between T2DM and cognitive function, and sets a precedence to examine age as a moderator when exploring relationships between T2DM and cognition in older adults. The current study also provides evidence that individuals with T2DM may have the ability to adapt to poorer glucose control and higher BMI, ameliorating any negative effects they might have on cognitive function in non-diabetics. Still, future studies are required to further understand and interpret the relationship between age and T2DM, as well as mechanisms by which cognitive function may be affected in T2DM populations.

5.0 BIBLIOGRAPHY

[1] H. Ahlgren, K. Henjum, O. P. Ottersen, and E. Runden-Pran. Validation of organotypical hippocampal slice cultures as an ex vivo model of brain ischemia: different roles of nmda receptors in cell death signalling after exposure to nmda or oxygen and glucose deprivation. *Cell Tissue Res*, 345(3):329–41, 2011.

[2] F. Amenta, F. Mignini, F. Rabbia, D. Tomassoni, and F. Veglio. Protective effect of anti-hypertensive treatment on cognitive function in essential hypertension: analysis of published clinical data. *J Neurol Sci*, 203-204:147–51, 2002.

[3] American Diabetes Association. Statistics about diabetes: Overall numbers, diabetes and prediabetes. http://www.diabetes.org/diabetes-basics/statistics/, 2016. Accessed: 2016-02-01.

[4] V. Anderson, C. Godfrey, J. V. Rosenfeld, and C. Catroppa. Predictors of cognitive function and recovery 10 years after traumatic brain injury in young children. *Pediatrics*, 129(2):e254–61, 2012.

[5] N. Awad, M. Gagnon, and C. Messier. The relationship between impaired glucose tolerance, type 2 diabetes, and cognitive function. *J Clin Exp Neuropsychol*, 26(8):1044–80, 2004.

[6] A. Baddeley. Working memory: The interface between memory and cognition. *J Cogn Neurosci*, 4(3):281–8, 1992.

[7] A. Baddeley. The fractionation of working memory. *Proc Natl Acad Sci U S A*, 93 (24):–72, 1996.

[8] W. A. Banks, J. B. Jaspan, and A. J. Kastin. Selective, physiological transport of insulin across the blood-brain barrier: novel demonstration by species-specific radioimmunoassays. *Peptides*, 18(8):1257–62, 1997.

[9] G. D. Baura, D. M. Foster, J. Porte, D., S. E. Kahn, R. N. Bergman, C. Cobelli, and M. W. Schwartz. Saturable transport of insulin from plasma into the central nervous system of dogs in vivo. a mechanism for regulated insulin delivery to the brain. *J Clin Invest*, 92(4):1824–30, 1993.

[10] G. J. Biessels and L. J. Kappelle. Increased risk of alzheimer's disease in type ii diabetes: insulin resistance of the brain or insulin-induced amyloid pathology? *Biochem Soc Trans*, 33(Pt 5):1041–4, 2005.

[11] G. J. Biessels, I. J. Deary, and C. M. Ryan. Cognition and diabetes: a lifespan perspective. *Lancet Neurol*, 7(2):184–90, 2008.

[12] R. J. Boado, J. Z. Lu, E. K. Hui, R. K. Sumbria, and W. M. Pardridge. Pharmacokinetics and brain uptake in the rhesus monkey of a fusion protein of arylsulfatase a and a monoclonal antibody against the human insulin receptor. *Biotechnol Bioeng*, 110(5): 1456–65, 2013.

[13] C. B. Brady, r. Spiro, A., and J. M. Gaziano. Effects of age and hypertension status on cognition: the veterans affairs normative aging study. *Neuropsychology*, 19(6):770–7, 2005.

[14] A. M. Brant, T. J. Jess, G. Milligan, C. M. Brown, and G. W. Gould. Immunological analysis of glucose transporters expressed in different regions of the rat brain and central nervous system. *Biochem Biophys Res Commun*, 192(3):–302, 1993.

[15] T. S. Braver, J. D. Cohen, L. E. Nystrom, J. Jonides, E. E. Smith, and D. C. Noll. A parametric study of prefrontal cortex involvement in human working memory. *Neuroimage*, 5(1):49–62, 1997.

[16] M. Brundel, M. van den Heuvel, J. de Bresser, L. J. Kappelle, and G. J. Biessels. Cerebral cortical thickness in patients with type 2 diabetes. *J Neurol Sci*, 299(1-2): 126–30, 2010.

[17] A. S. Buchman, R. S. Wilson, J. L. Bienias, R. C. Shah, D. A. Evans, and D. A. Bennett. Change in body mass index and risk of incident alzheimer disease. *Neurology*, 65(6):892–7, 2005.

[18] Centers for Disease Control and Prevention. *National diabetes statistics report: Estimates of diabetes and its Burden in the United States, 2014*. Department of Health and Human Services, Atlanta, GA, 2014.

[19] Y. Chen, Z. Liu, J. Zhang, K. Xu, S. Zhang, D. Wei, and Z. Zhang. Altered brain activation patterns under different working memory loads in patients with type 2 diabetes. *Diabetes Care*, 37(12):3157–63, 2014.

[20] S. R. Colberg, C. T. Somma, and S. R. Sechrist. Physical activity participation may offset some of the negative impact of diabetes on cognitive function. *J Am Med Dir Assoc*, 9(6):434–438, 2008.

[21] S. Colcombe and A. F. Kramer. Fitness effects on the cognitive function of older adults: a meta-analytic study. *Psychol Sci*, 14(2):125–30, 2003.

[22] R. J. Colman, R. M. Anderson, S. C. Johnson, E. K. Kastman, K. J. Kosmatka, T. M. Beasley, D. B. Allison, C. Cruzen, H. A. Simmons, J. W. Kemnitz, and R. Weindruch. Caloric restriction delays disease onset and mortality in rhesus monkeys. *Science*, 325 (5937):201–4, 2009.

[23] A. Convit. Links between cognitive impairment in insulin resistance: an explanatory model. *Neurobiol Aging*, 26 Suppl 1:31–5, 2005.

[24] A. R. Conway, M. J. Kane, M. F. Bunting, D. Z. Hambrick, O. Wilhelm, and R. W. Engle. Working memory span tasks: A methodological review and user's guide. *Psychon Bull Rev*, 12(5):769–786, 2005.

[25] S. Craft and G. S. Watson. Insulin and neurodegenerative disease: shared and specific mechanisms. *Lancet Neurol*, 3(3):169–78, 2004.

[26] A. K. Dahl and L. B. Hassing. Obesity and cognitive aging. *Epidemiol Rev*, 35:22–32, 2013.

[27] M. B. Davidson and D. L. Schriger. Effect of age and race/ethnicity on hba1c levels in people without known diabetes mellitus: implications for the diagnosis of diabetes. *Diabetes Res Clin Pract*, 87(3):415–21, 2010.

[28] S. M. de la Monte. Contributions of brain insulin resistance and deficiency in amyloid-related neurodegeneration in alzheimer's disease. *Drugs*, 72(1):49–66, 2012.

[29] R. A. DeFronzo and E. Ferrannini. Insulin resistance. a multifaceted syndrome responsible for niddm, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care*, 14(3):173–94, 1991.

[30] G. Desideri, C. Kwik-Uribe, D. Grassi, S. Necozione, L. Ghiadoni, D. Mastroiacovo, A. Raffaele, L. Ferri, R. Bocale, M. C. Lechiara, C. Marini, and C. Ferri. Benefits in cognitive function, blood pressure, and insulin resistance through cocoa flavanol consumption in elderly subjects with mild cognitive impairment: the cocoa, cognition, and aging (cocoa) study. *Hypertension*, 60(3):794–801, 2012.

[31] R. O. Dominguez, E. R. Marschoff, S. E. Gonzalez, M. G. Repetto, and J. A. Serra. Type 2 diabetes and/or its treatment leads to less cognitive impairment in alzheimer's disease patients. *Diabetes Res Clin Pract*, 98(1):68–74, 2012.

[32] K. R. Duffy and W. M. Pardridge. Blood-brain barrier transcytosis of insulin in developing rabbits. *Brain Res*, 420(1):32–8, 1987.

[33] H. Eichenbaum. Hippocampus: cognitive processes and neural representations that underlie declarative memory. *Neuron*, 44(1):109–20, 2004.

[34] M. F. Elias, P. K. Elias, L. M. Sullivan, P. A. Wolf, and R. B. D'Agostino. Lower

cognitive function in the presence of obesity and hypertension: the framingham heart study. *Int J Obes Relat Metab Disord*, 27(2):260–8, 2003.

[35] K. I. Erickson, M. W. Voss, R. S. Prakash, C. Basak, A. Szabo, L. Chaddock, J. S. Kim, S. Heo, H. Alves, S. M. White, T. R. Wojcicki, E. Mailey, V. J. Vieira, S. A. Martin, B. D. Pence, J. A. Woods, E. McAuley, and A. F. Kramer. Exercise training increases size of hippocampus and improves memory. *Proc Natl Acad Sci U S A*, 108(7):3017–22, 2011.

[36] M. A. Espeland, S. R. Rapp, G. A. Bray, D. K. Houston, K. C. Johnson, A. E. Kitabchi, A. L. Hergenroeder, J. Williamson, J. M. Jakicic, B. van Dorsten, S. B. Kritchevsky, M. Action for Health In Diabetes, S. Memory, and A. R. G. Look. Long-term impact of behavioral weight loss intervention on cognitive function. *J Gerontol A Biol Sci Med Sci*, 69(9):1101–8, 2014.

[37] M. A. Espeland, K. I. Erickson, R. H. Neiberg, J. Jakicic, T. A. Wadden, R. R. Wing, L. Desiderio, G. Erus, H. M, C. Davatzikos, B. J. Maschak-Carey, and P. J. Laurienti. Brain and white matter hyperintensity volumes after 10 years of random assignment to lifestyle intervention. *Diabetes Care*, 2016.

[38] L. G. Exalto, R. A. Whitmer, L. J. Kappele, and G. J. Biessels. An update on type 2 diabetes, vascular dementia and alzheimer's disease. *Exp Gerontol*, 47(11):858–64, 2012.

[39] K. Fabel, S. A. Wolf, D. Ehninger, H. Babu, P. Leal-Galicia, and G. Kempermann. Additive effects of physical exercise and environmental enrichment on adult hippocampal neurogenesis in mice. *Front Neurosci*, 3:50, 2009.

[40] M. E. Farmer, L. R. White, R. D. Abbott, S. J. Kittner, E. Kaplan, M. M. Wolz, J. A. Brody, and P. A. Wolf. Blood pressure and cognitive performance. the framingham study. *Am J Epidemiol*, 126(6):1103–14, 1987.

[41] S. L. Geijselaers, S. J. Sep, C. D. Stehouwer, and G. J. Biessels. Glucose regulation, cog-

nition, and brain mri in type 2 diabetes: a systematic review. *Lancet Diabetes Endocrinol*, 3(1):75–89, 2015.

[42] P. Gomez, R. Ratcliff, and M. Perea. A model of the go/no-go task. *J Exp Psychol Gen*, 136(3):389–413, 2007.

[43] M. M. Gonzales, T. Tarumi, S. C. Miles, H. Tanaka, F. Shah, and A. P. Haley. Insulin sensitivity as a mediator of the relationship between bmi and working memory-related brain activation. *Obesity (Silver Spring)*, 18(11):2131–7, 2010.

[44] C. E. Greenwood and G. Winocur. High-fat diets, insulin resistance and declining cognitive function. *Neurobiol Aging*, 26 Suppl 1:42–5, 2005.

[45] J. Havrankova, J. Roth, and M. J. Brownstein. Concentrations of insulin and insulin receptors in the brain are independent of peripheral insulin levels. studies of obese and streptozotocin-treated rodents. *J Clin Invest*, 64(2):636–42, 1979.

[46] W. H. Herman and R. M. Cohen. Racial and ethnic differences in the relationship between hba1c and blood glucose: implications for the diagnosis of diabetes. *J Clin Endocrinol Metab*, 97(4):1067–72, 2012.

[47] D. F. Hopkins and G. Williams. Insulin receptors are widely distributed in human brain and bind human and porcine insulin with equal affinity. *Diabet Med*, 14(12):1044–50, 1997.

[48] C. C. Hsu, M. L. Wahlqvist, M. S. Lee, and H. N. Tsai. Incidence of dementia is increased in type 2 diabetes and reduced by the use of sulfonylureas and metformin. *J Alzheimers Dis*, 24(3):485–93, 2011.

[49] D. F. Hultsch, C. Hertzog, B. J. Small, L. McDonald-Miszczak, and R. A. Dixon. Short-term longitudinal change in cognitive performance in later life. *Psychol Aging*, 7(4): 571–84, 1992.

[50] M. Jenkinson, P. Bannister, M. Brady, and S. Smith. Improved optimization for the robust and accurate linear registration and motion correction of brain images. *Neuroimage*, 17(2):825–41, 2002.

[51] C. Jongen, J. van der Grond, L. J. Kappelle, G. J. Biessels, M. A. Viergever, J. P. Pluim, and G. Utrecht Diabetic Encephalopathy Study. Automated measurement of brain and white matter lesion volume in type 2 diabetes mellitus. *Diabetologia*, 50(7):1509–16, 2007.

[52] C. R. Kahn. Insulin resistance, insulin insensitivity, and insulin unresponsiveness: a necessary distinction. *Metabolism*, 27(12 Suppl 2):1893–902, 1978.

[53] M. J. Kane, A. R. Conway, T. K. Miura, and G. J. Colflesh. Working memory, attention control, and the n-back task: a question of construct validity. *J Exp Psychol Learn Mem Cogn*, 33(3):615–22, 2007.

[54] A. Katsuki, Y. Sumida, E. C. Gabazza, S. Murashima, M. Furuta, R. Araki-Sasaki, Y. Hori, Y. Yano, and Y. Adachi. Homeostasis model assessment is a reliable indicator of insulin resistance during follow-up of patients with type 2 diabetes. *Diabetes Care*, 24 (2):362–5, 2001.

[55] L. Kerti, A. V. Witte, A. Winkler, U. Grittner, D. Rujescu, and A. Floel. Higher glucose levels associated with lower memory and reduced hippocampal microstructure. *Neurology*, 81(20):1746–52, 2013.

[56] B. Kim, L. L. McLean, S. S. Philip, and E. L. Feldman. Hyperinsulinemia induces insulin resistance in dorsal root ganglion neurons. *Endocrinology*, 152(10):3638–3647, 2011.

[57] A. F. Kramer and K. I. Erickson. Capitalizing on cortical plasticity: influence of physical activity on cognition and brain function. *Trends Cogn Sci*, 11(8):342–8, 2007.

[58] Z. Kroner. The relationship between alzheimer's disease and diabetes: Type 3 diabetes? *Altern Med Rev*, 14(4):373–9, 2009.

[59] S. Kullmann, S. Frank, M. Heni, C. Ketterer, R. Veit, H. U. Haring, A. Fritsche, and H. Preissl. Intranasal insulin modulates intrinsic reward and prefrontal circuitry of the human brain in lean women. *Neuroendocrinology*, 97(2):176–82, 2013.

[60] L. J. Launer, M. E. Miller, J. D. Williamson, R. M. Lazar, H. C. Gerstein, A. M. Murray, M. Sullivan, K. R. Horowitz, J. Ding, S. Marcovina, L. C. Lovato, J. Lovato, K. L. Margolis, P. O'Connor, E. W. Lipkin, J. Hirsch, L. Coker, J. Maldjian, J. L. Sunshine, C. Truwit, C. Davatzikos, R. N. Bryan, and A. M. investigators. Effects of intensive glucose lowering on brain structure and function in people with type 2 diabetes (accord mind): a randomised open-label substudy. *Lancet Neurol*, 10(11):969–77, 2011.

[61] R. L. Leckie, A. M. Weinstein, J. C. Hodzic, and K. I. Erickson. Potential moderators of physical activity on brain health. *J Aging Res*, 2012:948981, 2012.

[62] M. Lind, A. Pivodic, L. Cea-Soriano, O. Nerman, N. G. Pehrsson, and L. A. Garcia-Rodriguez. Changes in hba1c and frequency of measuring hba1c and adjusting glucose-lowering medications in the 10 years following diagnosis of type 2 diabetes: a population-based study in the uk. *Diabetologia*, 57(8):1586–94, 2014.

[63] A. R. G. Look, T. A. Wadden, D. S. West, L. Delahanty, J. Jakicic, J. Rejeski, D. Williamson, R. I. Berkowitz, D. E. Kelley, C. Tomchee, J. O. Hill, and S. Kumanyika. The look ahead study: a description of the lifestyle intervention and the evidence supporting it. *Obesity (Silver Spring)*, 14(5):737–52, 2006.

[64] D. R. Matthews, J. P. Hosker, A. S. Rudenski, B. A. Naylor, D. F. Treacher, and R. C. Turner. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*, 28(7):412–9, 1985.

[65] R. J. McCrimmon, C. M. Ryan, and B. M. Frier. Diabetes and cognitive dysfunction. *Lancet*, 379(9833):2291–9, 2012.

[66] B. S. McEwen and L. P. Reagan. Glucose transporter expression in the central nervous system: relationship to synaptic function. *Eur J Pharmacol*, 490(1-3):13–24, 2004.

[67] B. S. McEwen, L. Eiland, R. G. Hunter, and M. M. Miller. Stress and anxiety: structural plasticity and epigenetic regulation as a consequence of stress. *Neuropharmacology*, 62 (1):3–12, 2012.

[68] E. C. McNay, C. T. Ong, R. J. McCrimmon, J. Cresswell, J. S. Bogan, and R. S. Sherwin. Hippocampal memory processes are modulated by insulin and high-fat-induced insulin resistance. *Neurobiol Learn Mem*, 93(4):546–53, 2010.

[69] C. Messier. Impact of impaired glucose tolerance and type 2 diabetes on cognitive aging. *Neurobiol Aging*, 26 Suppl 1:26–30, 2005.

[70] L. A. Meusel, N. Kansal, E. Tchistiakova, W. Yuen, B. J. MacIntosh, C. E. Greenwood, and N. D. Anderson. A systematic review of type 2 diabetes mellitus and hypertension in imaging studies of cognitive aging: time to establish new norms. *Front Aging Neurosci*, 6:148, 2014.

[71] D. E. Moller and J. S. Flier. Insulin resistance–mechanisms, syndromes, and implications. *N Engl J Med*, 325(13):938–48, 1991.

[72] C. Moran, T. G. Phan, J. Chen, L. Blizzard, R. Beare, A. Venn, G. Munch, A. G. Wood, J. Forbes, T. M. Greenaway, S. Pearson, and V. Srikanth. Brain atrophy in type 2 diabetes: regional distribution and influence on cognition. *Diabetes Care*, 36(12):4036–42, 2013.

[73] M. E. Mortby, A. L. Janke, K. J. Anstey, P. S. Sachdev, and N. Cherbuin. High "normal" blood glucose is associated with decreased brain volume and cognitive performance in the 60s: the path through life study. *PLoS One*, 8(9):e73697, 2013.

[74] M. Munshi, L. Grande, M. Hayes, D. Ayres, E. Suhl, R. Capelson, S. Lin, W. Milberg, and K. Weinger. Cognitive dysfunction is associated with poor diabetes control in older adults. *Diabetes Care*, 29(8):1794–9, 2006.

[75] National Centers for Health Statistics. Deaths and mortality. http://www.cdc.gov/nchs/fastats/deaths.htm, 2014. Accessed: 2016-03-31.

[76] D. O'Malley, L. J. Shanley, and J. Harvey. Insulin inhibits rat hippocampal neurones via activation of atp-sensitive k+ and large conductance ca2+-activated k+ channels. *Neuropharmacology*, 44(7):855–63, 2003.

[77] A. M. Owen, K. M. McMillan, A. R. Laird, and E. Bullmore. N-back working memory paradigm: a meta-analysis of normative functional neuroimaging studies. *Hum Brain Mapp*, 25(1):46–59, 2005.

[78] P. Palta, A. L. Schneider, G. J. Biessels, P. Touradji, and F. Hill-Briggs. Magnitude of cognitive dysfunction in adults with type 2 diabetes: a meta-analysis of six cognitive domains and the most frequently reported neuropsychological tests within domains. *J Int Neuropsychol Soc*, 20(3):278–91, 2014.

[79] B. Paramo, K. Hernandez-Fonseca, A. M. Estrada-Sanchez, N. Jimenez, A. Hernandez-Cruz, and L. Massieu. Pathways involved in the generation of reactive oxygen and nitrogen species during glucose deprivation and its role on the death of cultured hippocampal neurons. *Neuroscience*, 167(4):1057–69, 2010.

[80] W. M. Pardridge, J. Eisenberg, and J. Yang. Human blood-brain barrier insulin receptor. *J Neurochem*, 44(6):1771–8, 1985.

[81] D. C. Park, G. Lautenschlager, T. Hedden, N. S. Davidson, A. D. Smith, and P. K. Smith. Models of visuospatial and verbal memory across the adult life span. *Psychol Aging*, 17(2):299–320, 2002.

[82] M. P. Parsons and M. Hirasawa. Atp-sensitive potassium channel-mediated lactate effect on orexin neurons: implications for brain energetics during arousal. *J Neurosci*, 30(24): 8061–70, 2010.

[83] N. V. Patel and C. E. Finch. The glucocorticoid paradox of caloric restriction in slowing brain aging. *Neurobiol Aging*, 23(5):707–17, 2002.

[84] N. V. Patel, M. N. Gordon, K. E. Connor, R. A. Good, R. W. Engelman, J. Mason, D. G. Morgan, T. E. Morgan, and C. E. Finch. Caloric restriction attenuates abeta-deposition in alzheimer transgenic models. *Neurobiol Aging*, 26(7):995–1000, 2005.

[85] L. Pellerin, A. K. Bouzier-Sore, A. Aubert, S. Serres, M. Merle, R. Costalat, and P. J. Magistretti. Activity-dependent regulation of energy metabolism by astrocytes: an update. *Glia*, 55(12):1251–62, 2007.

[86] L. C. Perlmuter, M. K. Hakami, C. Hodgson-Harrington, J. Ginsberg, J. Katz, D. E. Singer, and D. M. Nathan. Decreased cognitive function in aging non-insulin-dependent diabetic patients. *Am J Med*, 77(6):1043–8, 1984.

[87] J. J. Ratey and J. E. Loehr. The positive impact of physical activity on cognition during adulthood: a review of underlying mechanisms, evidence and recommendations. *Rev Neurosci*, 22(2):171–85, 2011.

[88] D. V. Rayner, M. E. Thomas, and P. Trayhurn. Glucose transporters (gluts 1-4) and their mrnas in regions of the rat brain: insulin-sensitive transporter expression in the cerebellum. *Can J Physiol Pharmacol*, 72(5):476–9, 1994.

[89] G. M. Reaven. Banting lecture 1988. role of insulin resistance in human disease. *Diabetes*, 37(12):1595–607, 1988.

[90] G. M. Reaven, L. W. Thompson, D. Nahum, and E. Haskins. Relationship between hyperglycemia and cognitive function in older niddm patients. *Diabetes Care*, 13(1): 16–21, 1990.

[91] C. K. Reuter-Lorenz, PA. Neurocognitive aging adn the compensation hypothesis. *Curr Dir Psychol Sci*, 17:177–182, 2008.

[92] G. S. Roth, D. K. Ingram, and M. A. Lane. Caloric restriction in primates and relevance to humans. *Ann N Y Acad Sci*, 928:305–15, 2001.

[93] C. M. Ryan and M. Geckle. Why is learning and memory dysfunction in type 2 diabetes limited to older adults? *Diabetes Metab Res Rev*, 16(5):308–15, 2000.

[94] C. M. Ryan, M. I. Freed, J. A. Rood, A. R. Cobitz, B. R. Waterhouse, and M. W. Strachan. Improving metabolic control leads to better working memory in adults with type 2 diabetes. *Diabetes Care*, 29(2):345–51, 2006.

[95] D. H. Ryan, M. A. Espeland, G. D. Foster, S. M. Haffner, V. S. Hubbard, K. C. Johnson, S. E. Kahn, W. C. Knowler, S. Z. Yanovski, and A. R. G. Look. Look ahead (action for health in diabetes): design and methods for a clinical trial of weight loss for the prevention of cardiovascular disease in type 2 diabetes. *Control Clin Trials*, 24(5):610–28, 2003.

[96] J. P. Ryan, D. F. Fine, and C. Rosano. Type 2 diabetes and cognitive impairment: contributions from neuroimaging. *J Geriatr Psychiatry Neurol*, 27(1):47–55, 2014.

[97] G. I. Shulman. Cellular mechanisms of insulin resistance. *J Clin Invest*, 106(2):171–6, 2000.

[98] N. Smania, R. Avesani, L. Roncari, P. Ianes, P. Girardi, V. Varalta, M. G. Gambini, A. Fiaschi, and M. Gandolfi. Factors predicting functional and cognitive recovery following severe traumatic, anoxic, and cerebrovascular brain damage. *J Head Trauma Rehabil*, 28(2):131–40, 2013.

[99] A. J. Sommerfield, I. J. Deary, V. McAulay, and B. M. Frier. Short-term, delayed, and working memory are impaired during hypoglycemia in individuals with type 1 diabetes. *Diabetes Care*, 26(2):390–6, 2003.

[100] P. J. Spauwen and C. D. Stehouwer. Cognitive decline in type 2 diabetes. *Lancet Diabetes Endocrinol*, 2(3):188–9, 2014.

[101] A. B. Steffens, A. J. Scheurink, J. Porte, D., and S. C. Woods. Penetration of peripheral glucose and insulin into cerebrospinal fluid in rats. *Am J Physiol*, 255(2 Pt 2):R200–4, 1988.

[102] R. P. Stolk, M. M. Breteler, A. Ott, H. A. Pols, S. W. Lamberts, D. E. Grobbee, and A. Hofman. Insulin and cognitive function in an elderly population. the rotterdam study. *Diabetes Care*, 20(5):792–5, 1997.

[103] A. M. Stranahan, E. D. Norman, K. Lee, R. G. Cutler, R. S. Telljohann, J. M. Egan, and M. P. Mattson. Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats. *Hippocampus*, 18(11):1085–8, 2008.

[104] J. D. Talbot, G. David, E. F. Barrett, and J. N. Barrett. Calcium dependence of damage to mouse motor nerve terminals following oxygen/glucose deprivation. *Exp Neurol*, 234 (1):95–104, 2012.

[105] K. Talbot, H. Y. Wang, H. Kazi, L. Y. Han, K. P. Bakshi, A. Stucky, R. L. Fuino, K. R. Kawaguchi, A. J. Samoyedny, R. S. Wilson, Z. Arvanitakis, J. A. Schneider, B. A. Wolf, D. A. Bennett, J. Q. Trojanowski, and S. E. Arnold. Demonstrated brain insulin resistance in alzheimer's disease patients is associated with igf-1 resistance, irs-1 dysregulation, and cognitive decline. *J Clin Invest*, 122(4):1316–38, 2012.

[106] A. M. Tiehuis, K. L. Vincken, E. van den Berg, J. Hendrikse, S. M. Manschot, W. P. Mali, L. J. Kappelle, and G. J. Biessels. Cerebral perfusion in relation to cognitive function and type 2 diabetes. *Diabetologia*, 51(7):1321–6, 2008.

[107] F. G. Toledo, E. V. Menshikova, V. B. Ritov, K. Azuma, Z. Radikova, J. DeLany, and D. E. Kelley. Effects of physical activity and weight loss on skeletal muscle mitochondria and relationship with glucose control in type 2 diabetes. *Diabetes*, 56(8):2142–2147, 2007.

[108] D. Umpierre, P. A. Ribeiro, C. K. Kramer, C. B. Leitao, A. T. Zucatti, M. J. Azevedo, J. L. Gross, J. P. Ribeiro, and B. D. Schaan. Physical activity advice only or structured exercise training and association with hba1c levels in type 2 diabetes: a systematic review and meta-analysis. *JAMA*, 305(17):1790–9, 2011.

[109] J. W. Unger, J. N. Livingston, and A. M. Moss. Insulin receptors in the central nervous

system: localization, signalling mechanisms and functional aspects. *Prog Neurobiol*, 36 (5):343–62, 1991.

[110] N. T. Vagelatos and G. D. Eslick. Type 2 diabetes as a risk factor for alzheimer's disease: the confounders, interactions, and neuropathology associated with this relationship. *Epidemiol Rev*, 35:152–60, 2013.

[111] E. van den Berg, R. P. Kloppenborg, R. P. Kessels, L. J. Kappelle, and G. J. Biessels. Type 2 diabetes mellitus, hypertension, dyslipidemia and obesity: A systematic comparison of their impact on cognition. *Biochim Biophys Acta*, 1792(5):470–81, 2009.

[112] M. Vanhanen, K. Koivisto, J. Kuusisto, L. Mykkanen, E. L. Helkala, T. Hanninen, S. Riekkinen, P., H. Soininen, and M. Laakso. Cognitive function in an elderly population with persistent impaired glucose tolerance. *Diabetes Care*, 21(3):398–402, 1998.

[113] T. A. Wadden, R. H. Neiberg, R. R. Wing, J. M. Clark, L. M. Delahanty, J. O. Hill, J. Krakoff, A. Otto, D. H. Ryan, M. Z. Vitolins, and A. R. G. Look. Four-year weight losses in the look ahead study: factors associated with long-term success. *Obesity (Silver Spring)*, 19(10):1987–98, 2011.

[114] P. S. Walker, J. A. Donovan, B. G. Van Ness, R. E. Fellows, and J. E. Pessin. Glucose-dependent regulation of glucose transport activity, protein, and mrna in primary cultures of rat brain glial cells. *J Biol Chem*, 263(30):15594–601, 1988.

[115] B. J. Wallum, J. Taborsky, G. J., J. Porte, D., D. P. Figlewicz, L. Jacobson, J. C. Beard, W. K. Ward, and D. Dorsa. Cerebrospinal fluid insulin levels increase during intravenous insulin infusions in man. *J Clin Endocrinol Metab*, 64(1):190–4, 1987.

[116] D. Weschsler. *Wechsler memory scale (WMS-IV)*. The Psychological Corporation, New York, 2009.

[117] R. K. West, R. Ravona-Springer, J. Schmeidler, D. Leroith, K. Koifman, E. Guerrero-Berroa, R. Preiss, H. Hoffman, J. M. Silverman, A. Heymann, and M. Schnaider-Beeri.

The association of duration of type 2 diabetes with cognitive performance is modulated by long-term glycemic control. *Am J Geriatr Psychiatry*, 22(10):1055–9, 2014.

[118] D. S. Woodruff-Pak, M. R. Foy, G. G. Akopian, K. H. Lee, J. Zach, K. P. Nguyen, D. M. Comalli, J. A. Kennard, A. Agelan, and R. F. Thompson. Differential effects and rates of normal aging in cerebellum and hippocampus. *Proc Natl Acad Sci U S A*, 107 (4):1624–9, 2010.

[119] World Health Organization. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: Report of a WHO/IDF consultation, 2006.

[120] K. Yaffe, T. Blackwell, R. A. Whitmer, K. Krueger, and E. Barrett Connor. Glycosylated hemoglobin level and development of mild cognitive impairment or dementia in older women. *J Nutr Health Aging*, 10(4):293–5, 2006.

[121] M. A. Yassa, A. T. Mattfeld, S. M. Stark, and C. E. Stark. Age-related memory deficits linked to circuit-specific disruptions in the hippocampus. *Proc Natl Acad Sci U S A*, 108 (21):8873–8, 2011.

[122] Y. Zhang, S. Lu, C. Liu, H. Zhang, X. Zhou, C. Ni, W. Qin, and Q. Zhang. Altered brain activation and functional connectivity in working memory related networks in patients with type 2 diabetes: An ica-based analysis. *Sci Rep*, 6:23767, 2016.